OLYMPUS®

Quick Start

FLUOVIEW FV1000

CONFOCAL LASER SCANNING BIOLOGICAL MICROSCOPE

FV10-ASW【Ver1.3】

Petition

Thank you for your purchase of Olympus microscope at this time.
Prior to using this microscope, read this instruction manual for sure to utilize full-fledged performance of this microscope and ensure customer's safety.
In addition, read section "Safety Guide" and "Hardwarel" of User's Manual – FLUOVIEW FV1000 as well as instruction manual for microscope to understand how to use the equipment thoroughly.
To use laser system correctly, read instruction manuals that come with each laser equipment and light power system.
Hold this manual by your side when using this microscope all the time and keep it with care after reading.

AX7284

Caution

1. Part or whole of this software as well as manual shall not be used or duplicated without consent.
2. Contents described in this manual are subject to change without notice in future.

Registered trademark

OLYMPUS, FLUOVIEW and LSM are of our registered trademark.

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1 About this manual

This manual describes about windows that can be displayed (Section 2) and simple operation procedures (Section 3).

For further details of each window, see Online Help that appears through [Help] – [Help].

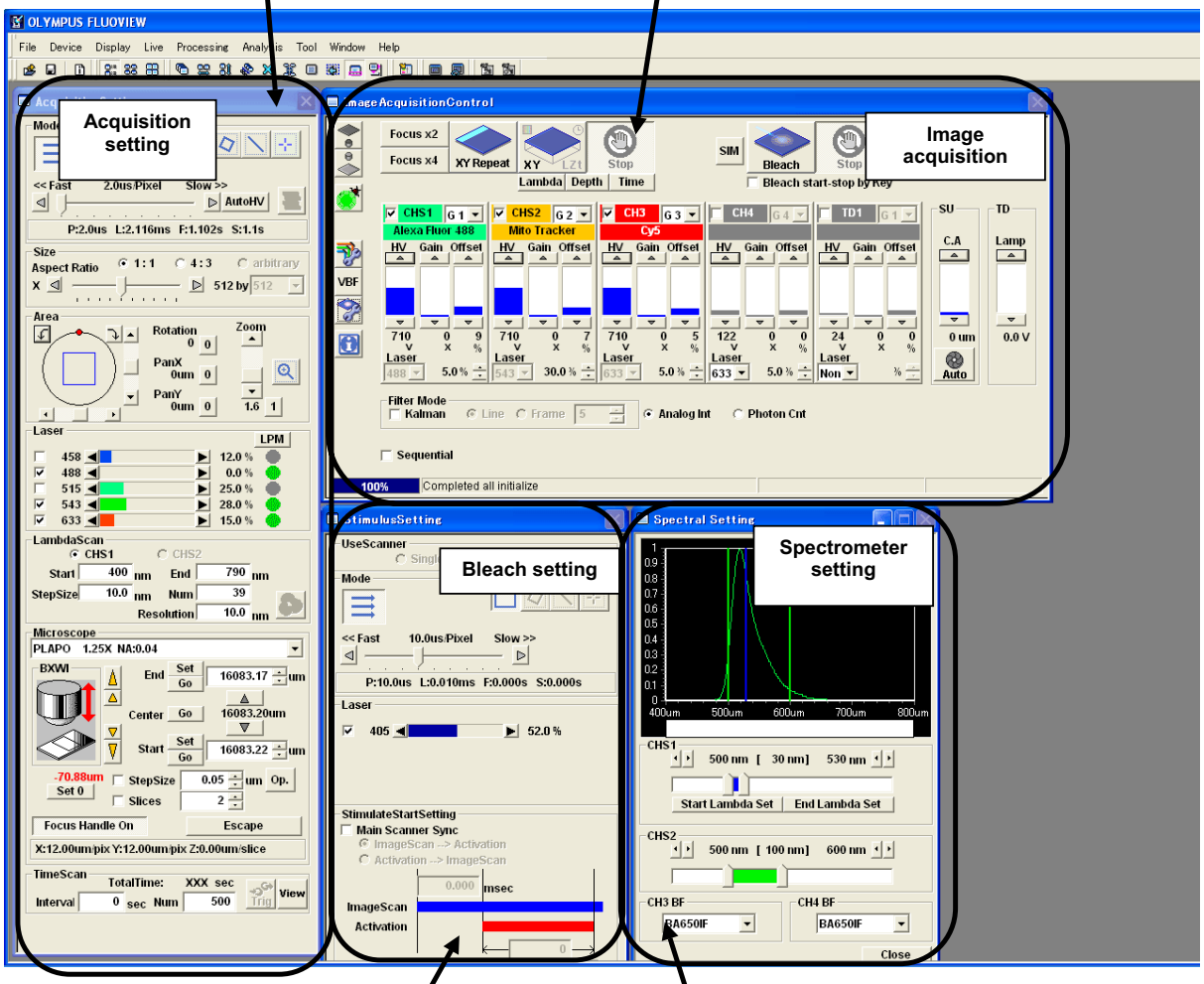
2 Windows in this software

Window used on FV10-ASW (Application Software) is individually introduced.

Windows in this software

Window to set image acquisition

Window to acquire image

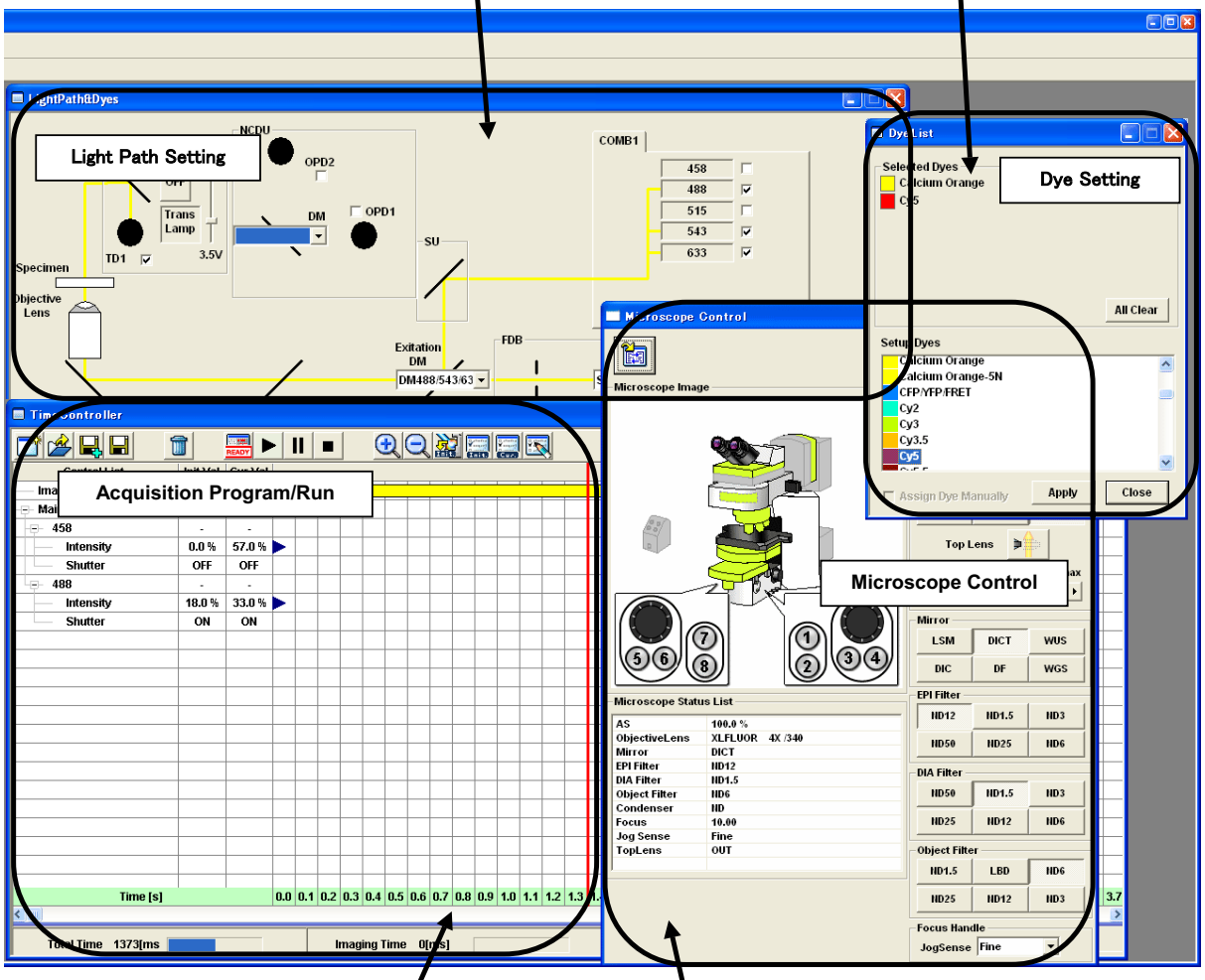


Window that opens when button for "Image Acquisition" is pressed and it is used for bleach setting.

Window that opens when button VBF for "Image Acquisition" is pressed and it is used for spectrometer setting.

Window that opens when button for "Image Acquisition" is pressed and it is used for light path setting.

Window that opens when button for "Image Acquisition" is pressed and it is used for Dye setting.



[Device] – [Time Controller]

Window that the acquisition is programmed and the process is executed.

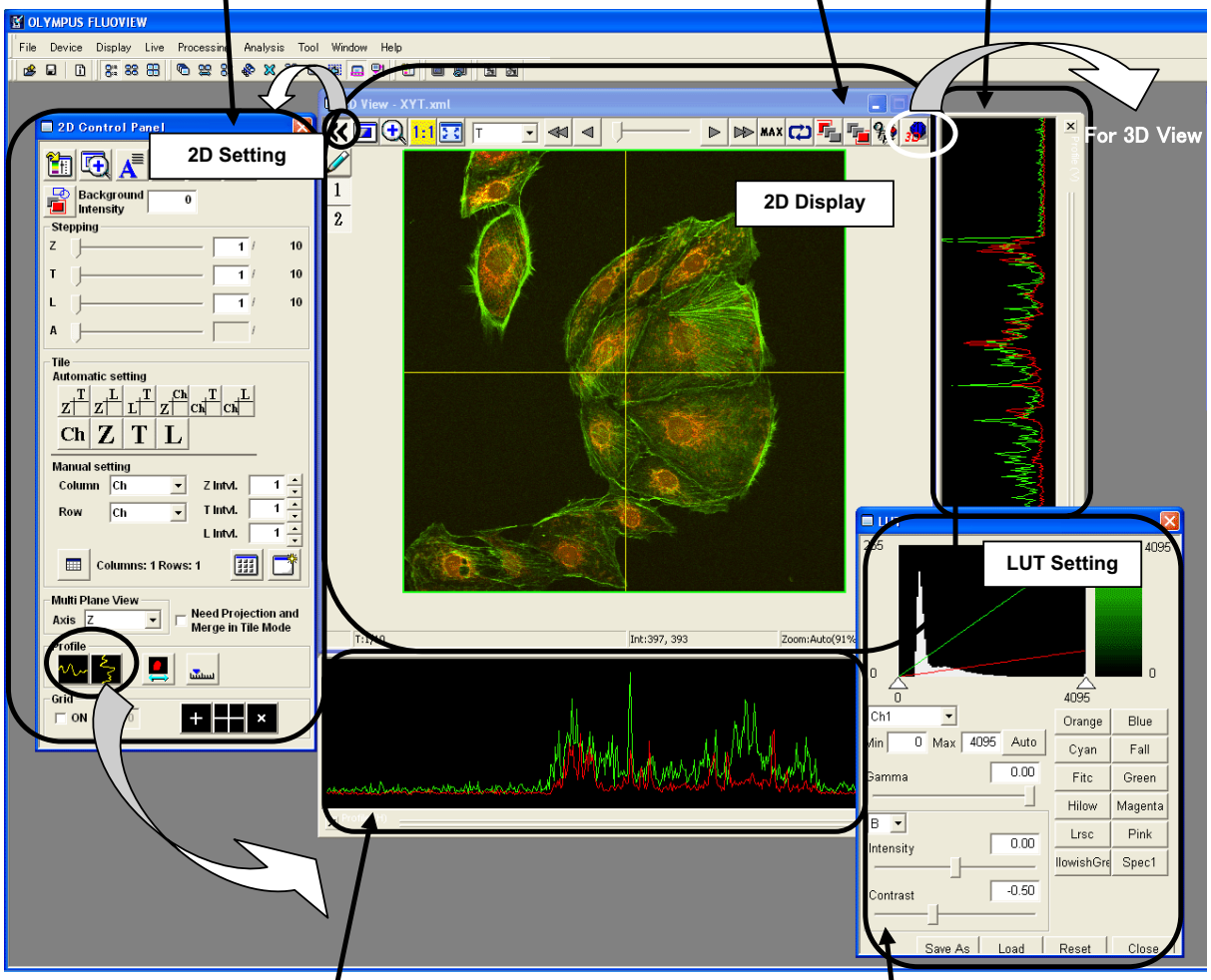
[Device] - [Microscope Controller]

Window to control microscope main unit and motorized section attached to the microscope main unit.

Window that opens when button for "2D Display" is pressed and it is used for 2D display setting.

Window to display 2D. When "Live View" appears, the image being acquired will be displayed.

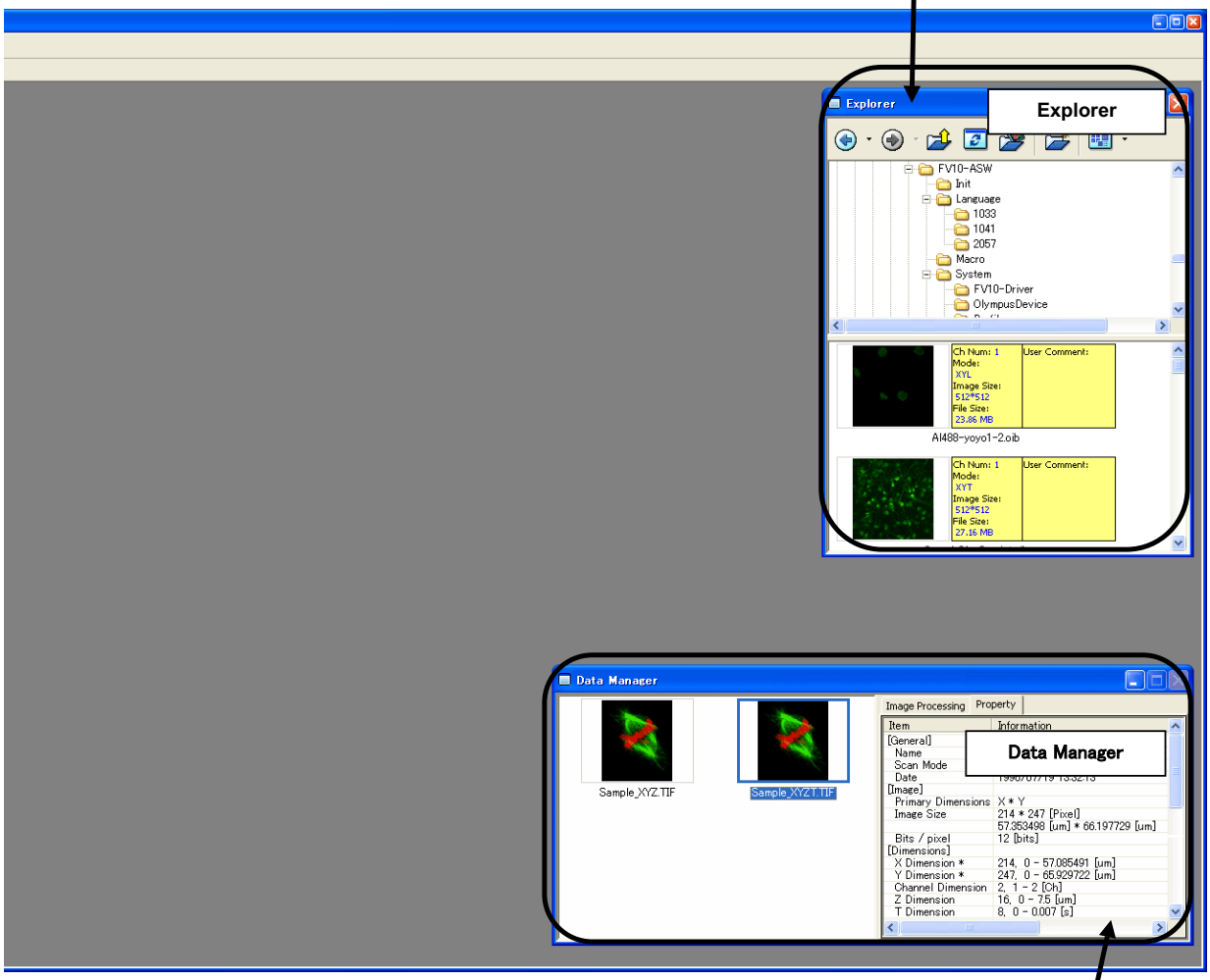
Window that opens when button for "2D Setting" is pressed and it is used to display intensity profile of 2D image in vertical direction.



Window that opens when button for "2D Setting" is pressed and it is used to display intensity profile of 2D image in horizontal direction.

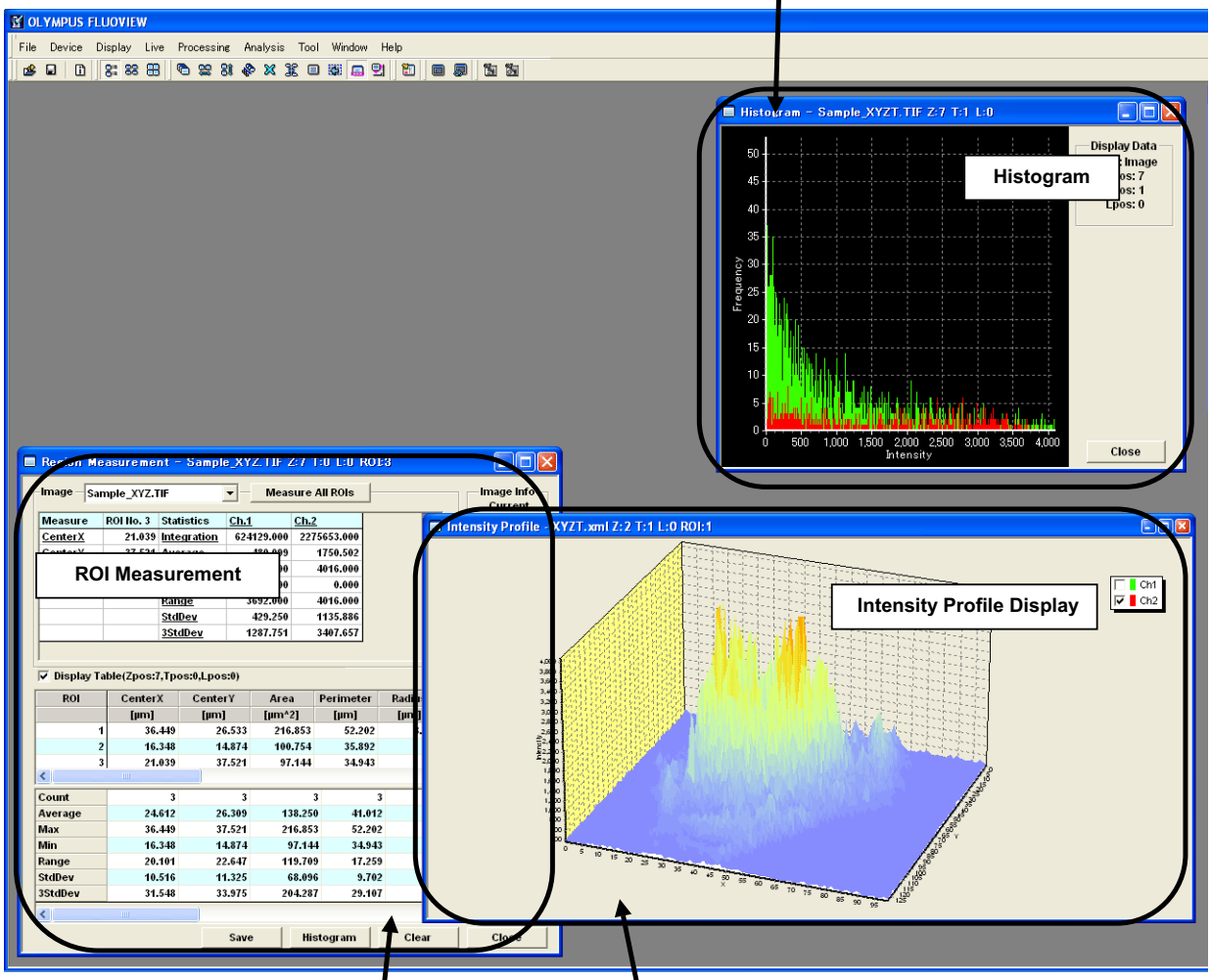
Window that opens when button for "2D Setting" is pressed and it is used for LUT setting that changes image display color.

Window to load an image by selecting folder that saves the images. When thumbnail image is double-clicked, the file is called and displayed.



Window where thumbnail and property of the image currently loaded is displayed.

[Analysis] – [Single] – [Histogram]
Window that Histogram is displayed.

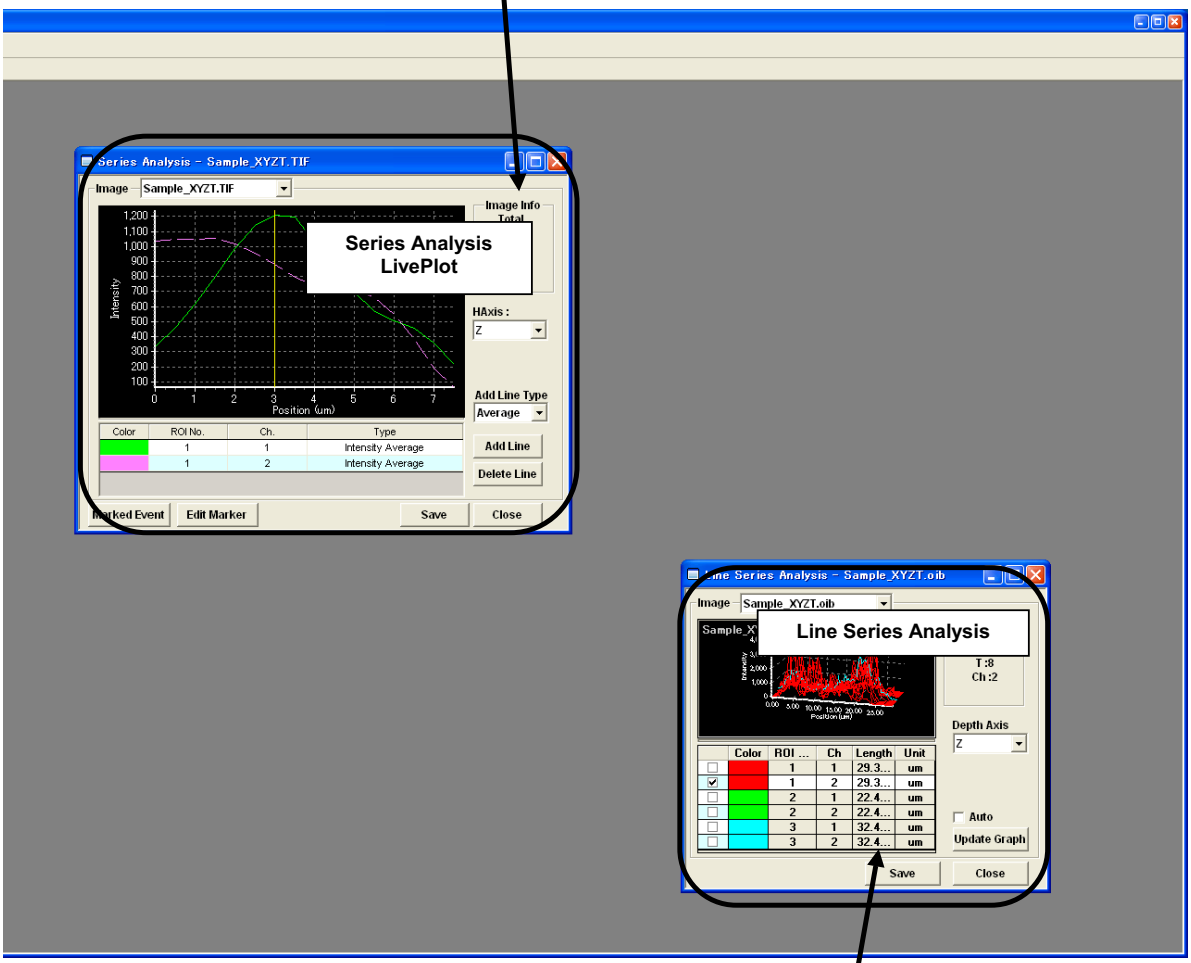


[Analysis] - [Single]—[Measurement]
Window that ROI (Region of Interest) is measured.

[Analysis] – [Single] – [Intensity Profile]
Window that intensity profile inside ROI selected is displayed.

[Analysis] - [Series]

Window where series image analysis is done. Time transition for Average and Integration value, etc., can be displayed in graph. Setting the applicable image to "Live" causes the graph to appear in real-time.



[Analysis] – [Line Series]

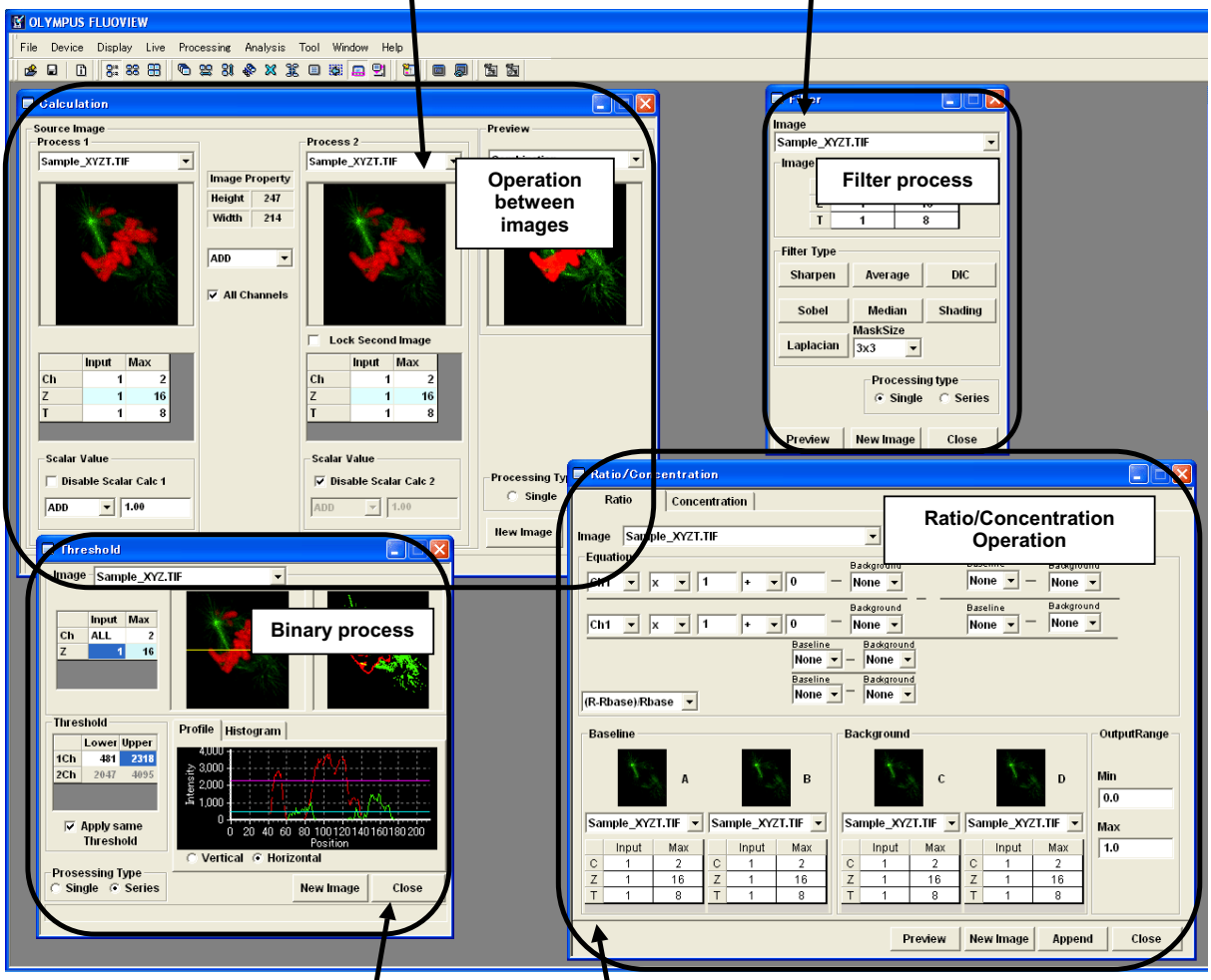
Window used to perform series image analysis on ROI (Region of Interest) of line.

Windows in this software

[Processing] – [Image Calculation]

Window used to perform operations between images. Logic and arithmetic operation for image intensity can be done.

[Processing] - [Filter setting]
Window to enhance the image.



[Processing] – [Threshold]

Window to turn the image to binary data.

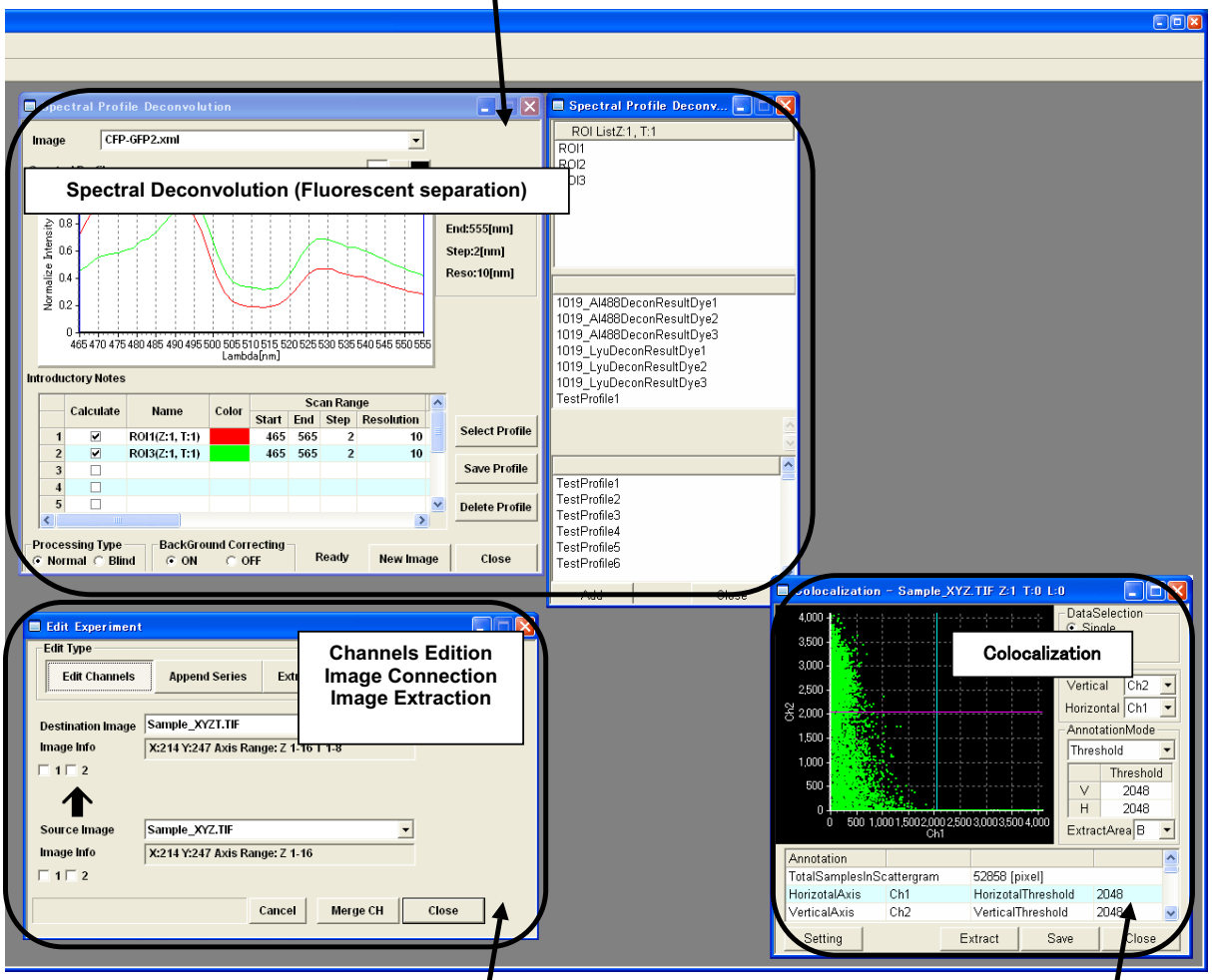
[Processing] - [Ratio/Concentration]

1.Image of intensity ratio between channels converted to intensity value is created.

2.Image of ion concentration of specimen converted to intensity value is created.

[Processing] – [Spectral Deconvolution]

Window for spectral deconvolution (the process to separate fluorescent from image of multi-dyeing specimen).



[Processing] - [Edit Experiment]

1.Channel composition/extraction can be done.

2.Image can be connected each other.

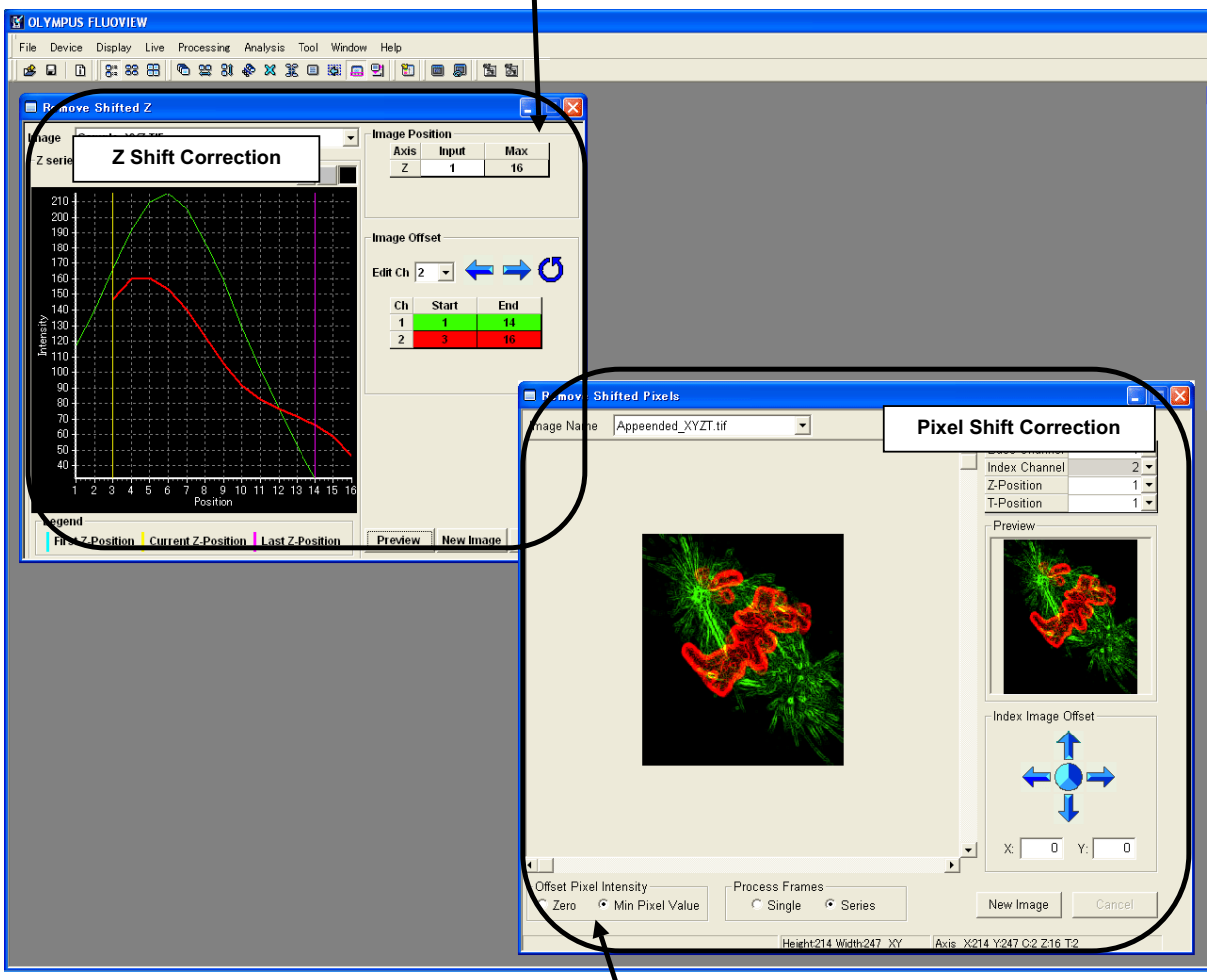
3.Image can be extracted.

[Processing] - [Colocalization]

It is the window to see how the intensity is overlapping.

For example, it is possible to see what extent the bright area of Ch1 is overlapping with the bright area of Ch2 quantitatively.

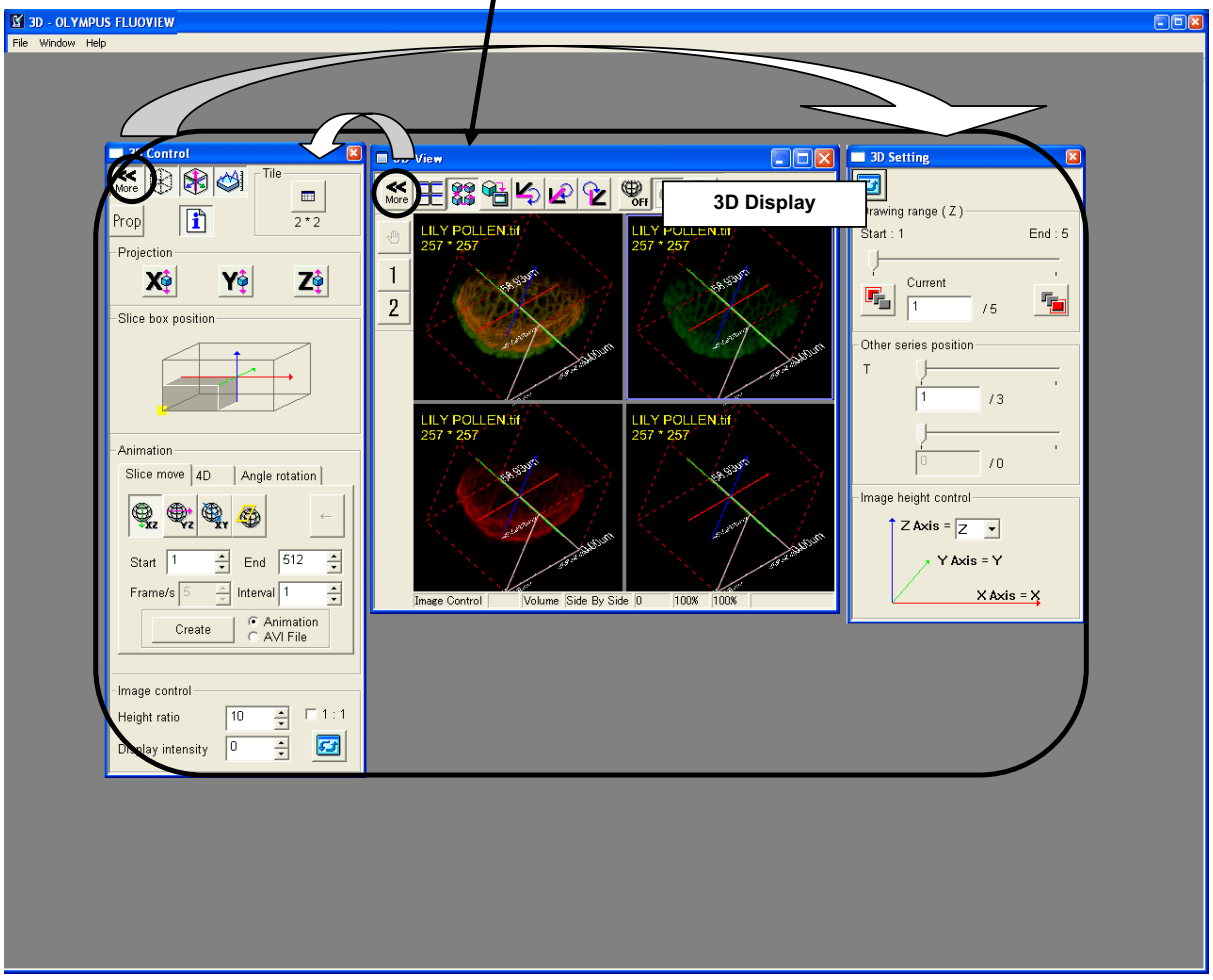
[Processing] – [Correcting Z Gaps]
Window where Z position shift between image channels is corrected.



[Processing] - [Correcting Pixel Gaps]
Window where pixel position is corrected in XY direction, if it is shifted, between light receiving channels while image is being acquired.

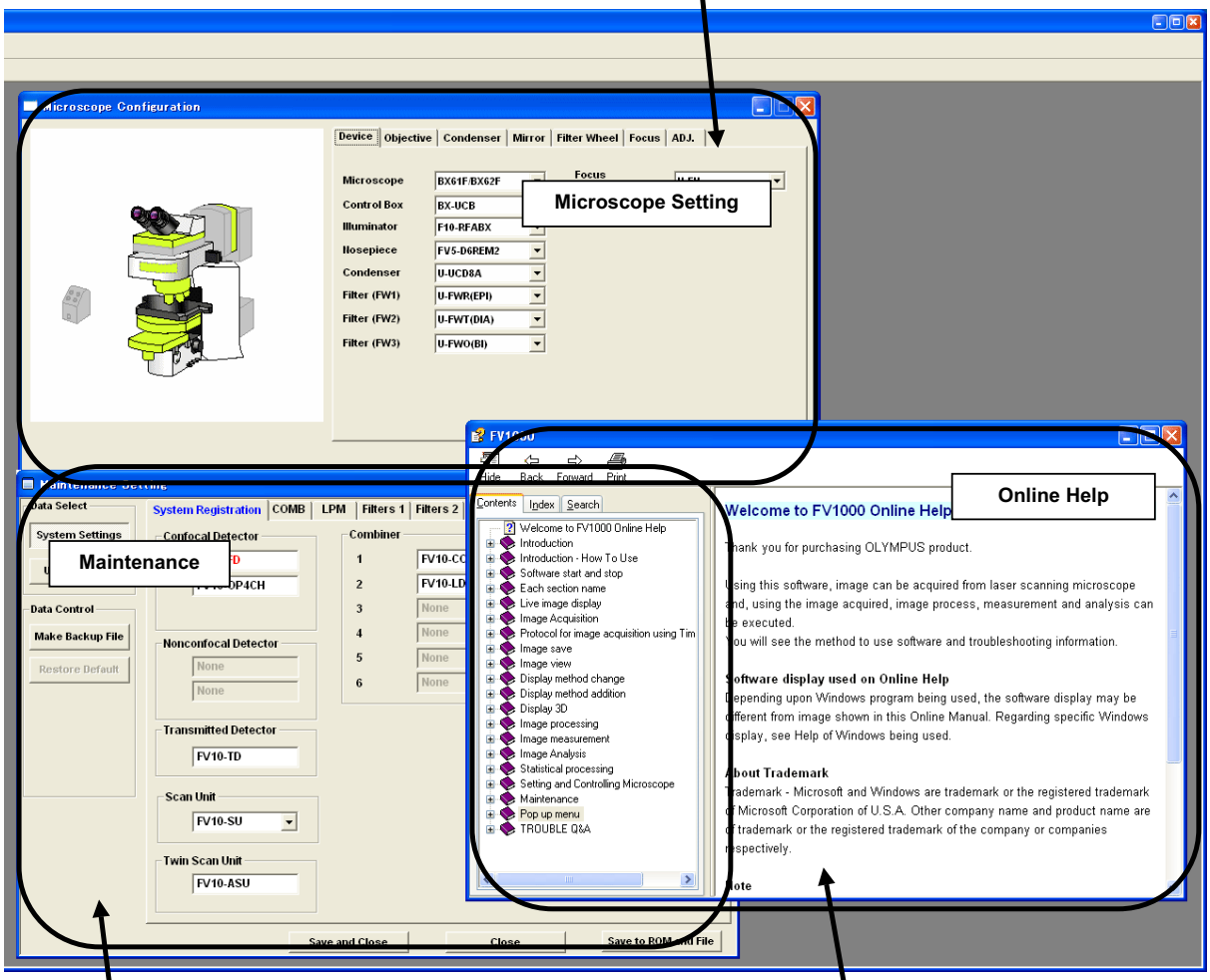
Window for 3D display that opens

when 2D Display **button is pressed.**



[Tool] - [Microscope Configuration]

Window for setting microscope main unit and motorized section attached to microscope main unit.



[Tool] – [Maintenance]

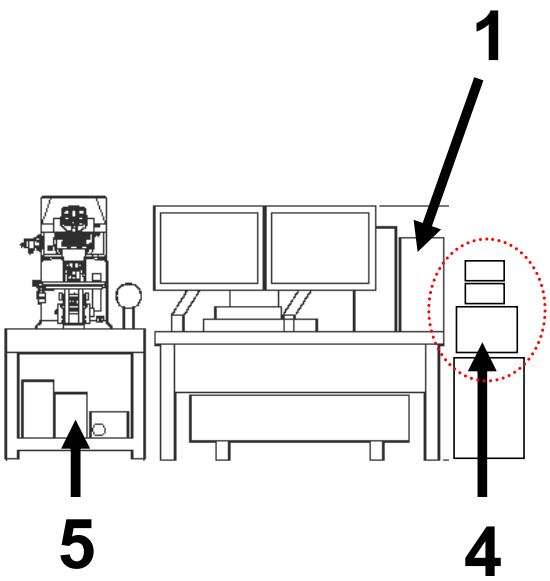
Maintenance of system can be done.

[Help] – [Help]

Online Help. See here for the details of operation methods.

3 Basic operation procedures

3.1.System Start Up



1. Turn computer to ON.
2. Turn FV10-PSU to ON.
3. Turn BX-UCB or IX2-UCB to ON.
4. Turn laser to ON.
   (Turn key switch.)
   4-1. Multi Ar
   (458nm・488nm・514nm) ON
   4-2. HeNe(G)(543nm) ON
   4-3. HeNe(R)(633nm) ON
5. Turn mercury lamp power to ON.
6. Enter user name/password and logon to WindowsXP.

   User name: Administrator
   Password: fluoview

7. Double-click

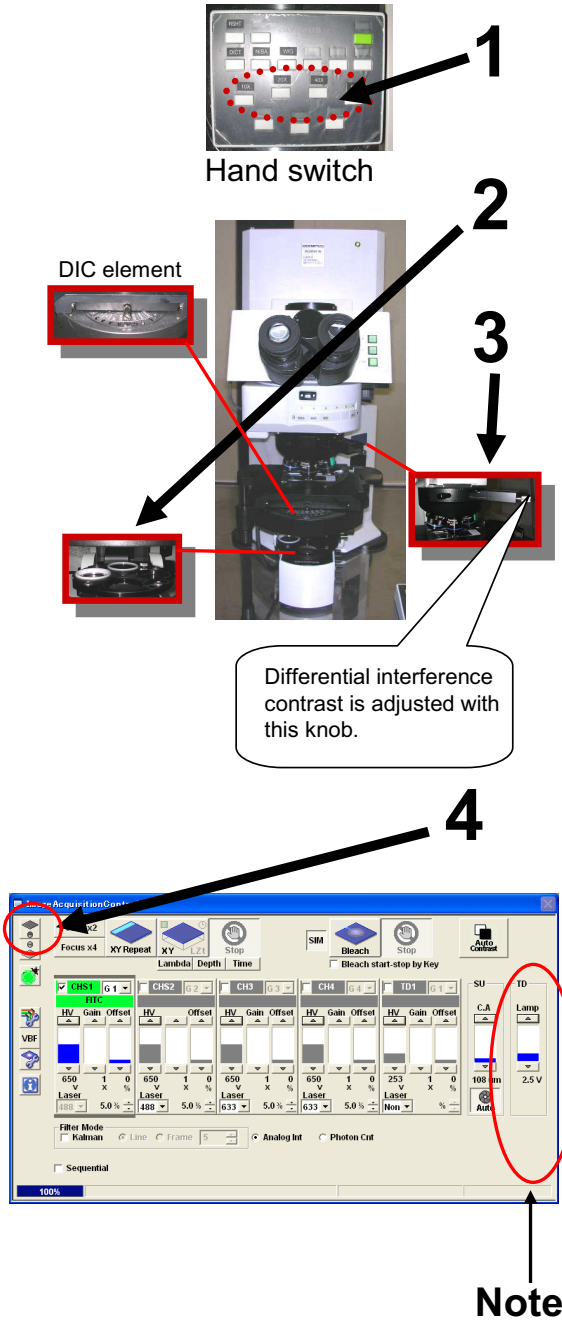
and FV10-ASW software starts up.

   User name: Administrator
   Password: Administrator

* It takes a certain period of time for start up.

3.2.Visual observation with microsocpe (Differential Interference Image Observation)

■ ■ Erecting type BX ■ ■



1. Select objective lens with hand switch. (Ref: ■Memo■）

2. Insert polarizer.

3. Insert differential interference prism slider.

4. Click of FV10-ASW software.

Note 1: Light of halogen lamp is adjusted, using TD Lamp slider on FV10-ASW software.

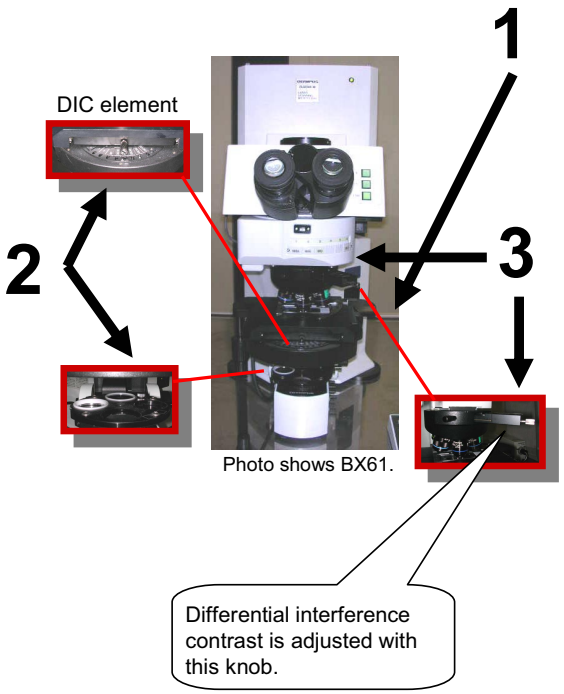
Note 2: Check if the filter turret is set to 6. DIC. If not, press DICT button of hand switch.

5. Adjust focus.

■ Memo ■
When magnification change is required hereafter, do procedure 1 only and the followings will be changed accordingly:;
- Objective lens
- Optimum DIC element to each objective lens

3.3.Visual observation with microscope (Differential Interference Image Observation)

■ ■ Erecting type BXWI ■ ■

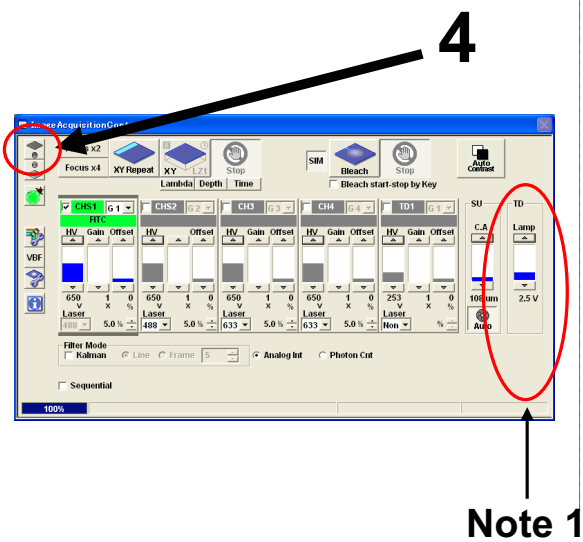


1. Select objective lens.

2. Insert polarizer and select DIC element.

3. Insert differential interference prism slider and select differential interference analyzer (DICT).

4. Click of FV10-ASW software.



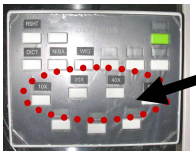
Note 1: Light of halogen lamp is adjusted, using TD Lamp slider on FV10-ASW software.

Note 2: Check if the filter turret is set to 6. DICT. If not, press DICT button of hand switch.

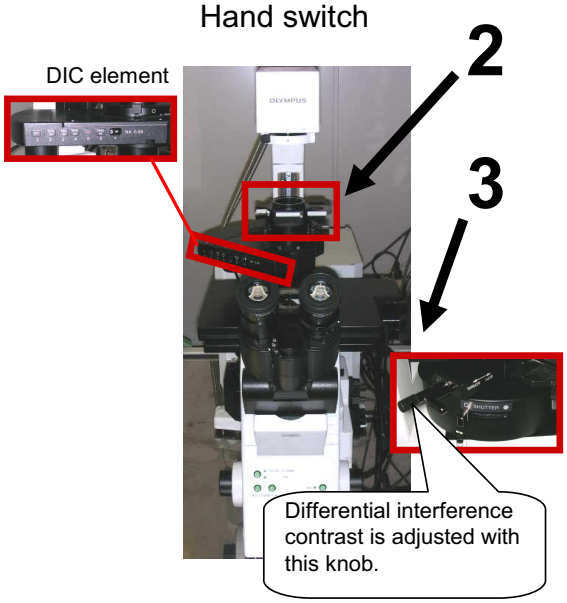
5. Adjust focus.

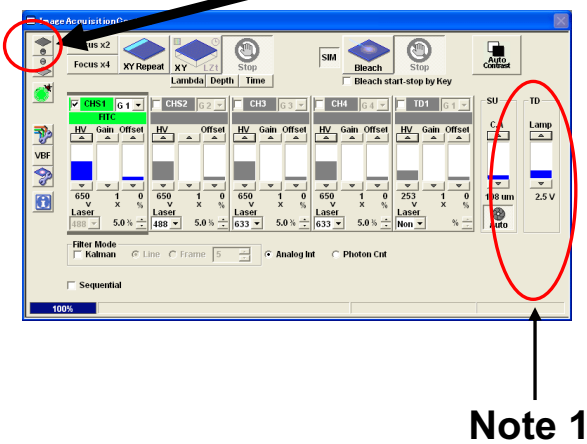
3.4.Visual observation with microscope (Differential Interference Image Observation)

■ ■ Inverted type IX ■ ■



Hand switch





1. Select objective lens with hand switch. (Ref: ■Memo■)
2. Insert polarizer.
3. Insert differential interference prism slider.
4. Click of FV10-ASW software.

Note 1: Light of halogen lamp is adjusted, using TD Lamp slider on FV10-ASW software.

Note 2: Check if the filter turret is set to 6. DIC. If not, press DICT button of hand switch.

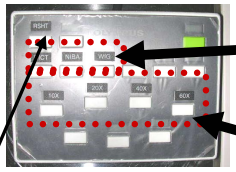
5. Adjust focus.

■ Memo ■

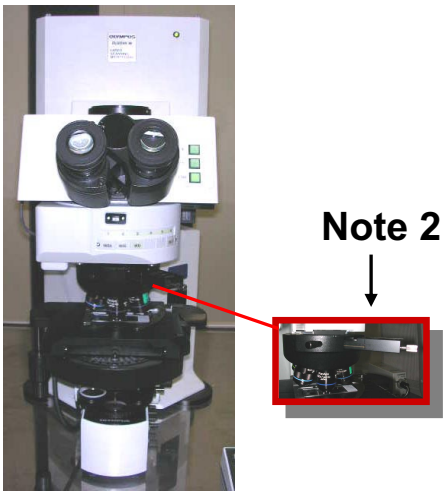
When magnification change is required hereafter, do procedure 1 only and the followings will be changed accordingly;
- Objective lens
- Optimum DIC element for each objective lens

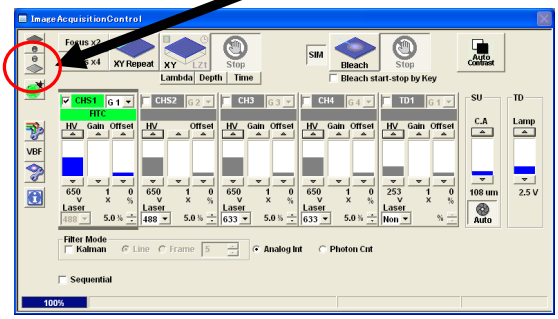
3.5.Visual observation with microscope (Fluorescent image observation)

■ ■ Erecting type BX, BXWI ■ ■



Note 1





1. Select objective lens with hand switch.

2. Click of FV10-ASW software.

Note 1: Operation in procedure 2 turns the mode to fluorescent visual mode.
At this moment, mechanical shutter of mercury lamp will open. Be careful.
(Mercury lamp shutter – Close is done from hand switch.)
Note 2: Verify that the differential interference slider is pulled out.

3. Select fluorescent filter with hand switch. (Ref: ■Memo■)

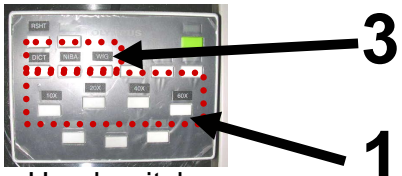
■ Memo ■
About fluorescent filter

NIBA: Blue excitation/Green fluorescent
(Example: FITC, EGFP etc.)
WIG: Green excitation/Red fluorescent
(Example: Rhodamine, DsRed. etc.)

4. Adjust focus.

3.6.Visual observation with microscope (Fluorescent image observation)

■ ■ Inverted type IX ■ ■



Hand switch

Note 2

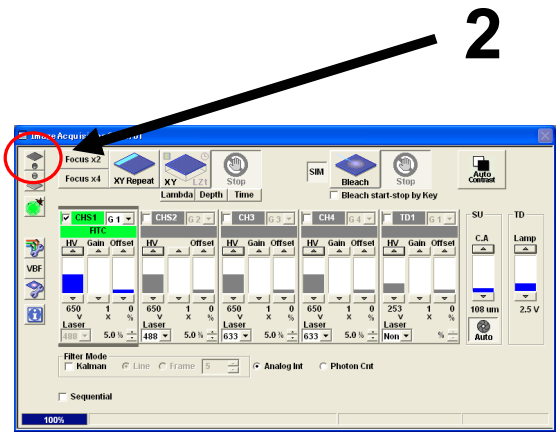
Note 1 & 3

1. Select objective lens with hand switch.

2. Click of FV10-ASW software.

Note 1: Operation in procedure 3 turns the mode to fluorescent visual mode.
At this moment, mercury lamp mechanical shutter will open. Be careful.
(It is recommended that the mercury lamp manual shutter is set to Close ● beforehand.)
Note 2: Verify that the differential interference slider is pulled out.

3. Select fluorescent filter with hand switch. (Ref: ■Memo■)



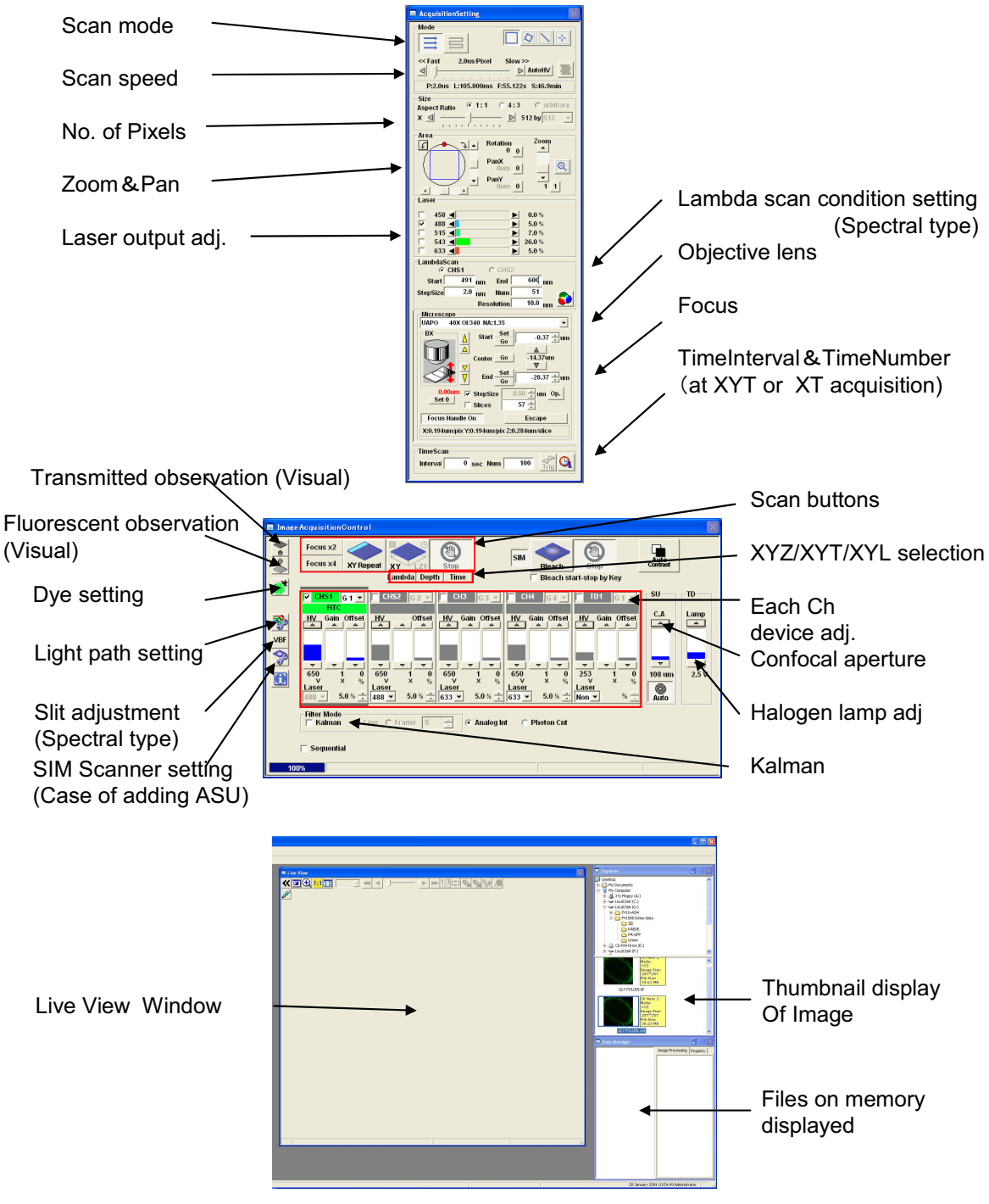
Note 3: When viewing through microscope, verify whether or not the mercury lamp mechanical shutter is set to Open ○.

4. Adjust focus.

■ Memo ■
About fluorescent filter

NIBA: Blue excitation/Green fluorescent
(Example: FITC, EGFP, etc.)
WIG : Green excitation/Red fluorescent
(Example: Rhodamine, DsRed, etc.)

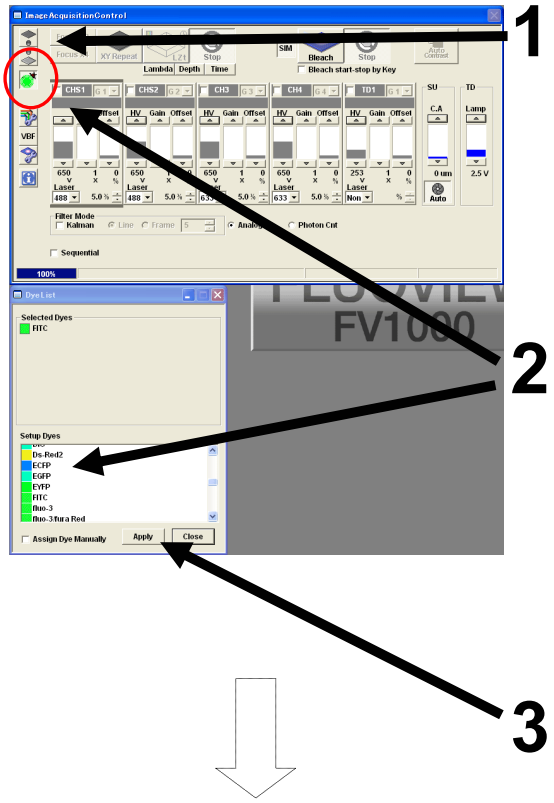
3.7.Image acquisition panel - Outline

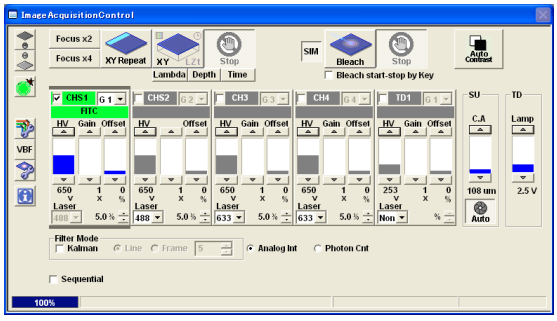


3.8.Image acquisition (Single dyeing color XY)

■■ 1 slice of image acquisition (XY plane) (Fluorescent image only)■■

Example: Single dyeing with green fluorescence (FITC)





Window after DyeApply is clicked

1. Click of FV10-ASW software and set it to “not pressed” state and close shutter of fluorescent lamp.

   Click and set it to “not pressed” state and close shutter of halogen lamp.

2. Click DyeList button and double-click fluorescent reagent (FITC) that should be observed from DyeList panel.

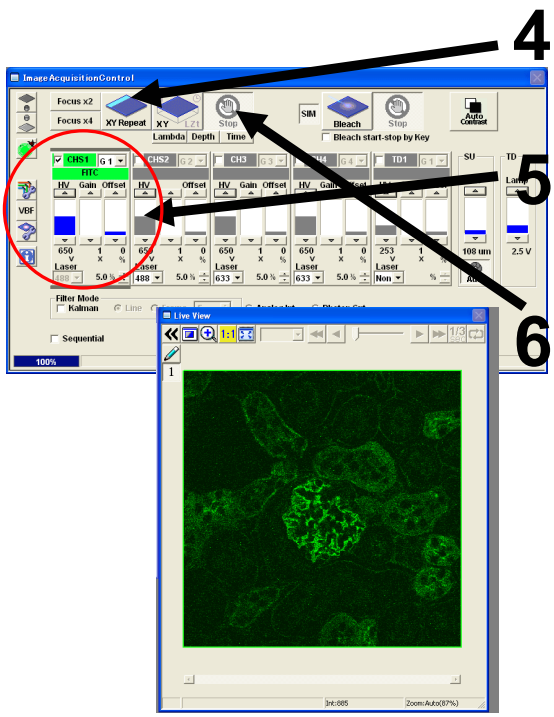
   * When reselecting, double-click the fluorescent dye in AssignDyes one time to clear it, and then, perform operation in procedure 2.

3. Click Apply button.

   (DyeList panel can be closed with、Close button.)

- In case of Spectral type -
When this operation is done, the fluorescent wavelength suit to Fluorescent Dye selected is defined.

Moreover, the fluorescent wavelength is manually changeable.
For further details, see Annex 3.



4. Click XY Repeat button to perform scanning.

5. Adjust green (FITC) image. (See below for outline of image adjustment. For further details, see Annex 1.)

6. Click STOP button to stop scanning. (Ref: ■Memo■)

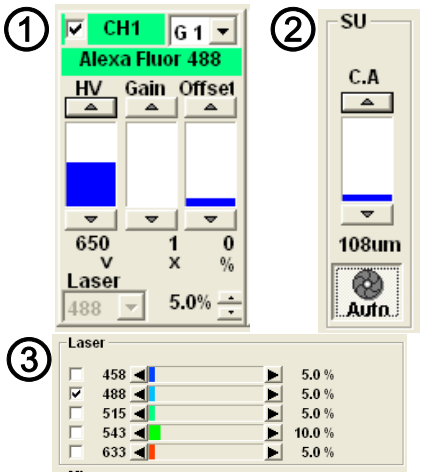
■ Memo■
About scan button

: Consecutive scan

: Scan stop

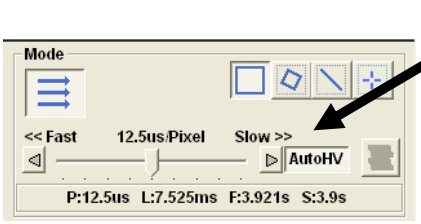
: Rough scan
(Lines skipped and scanned)

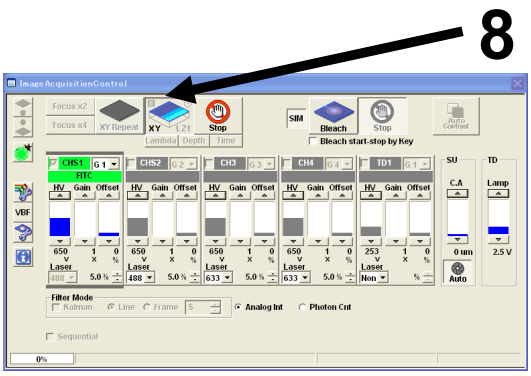
Outline of image adjustment

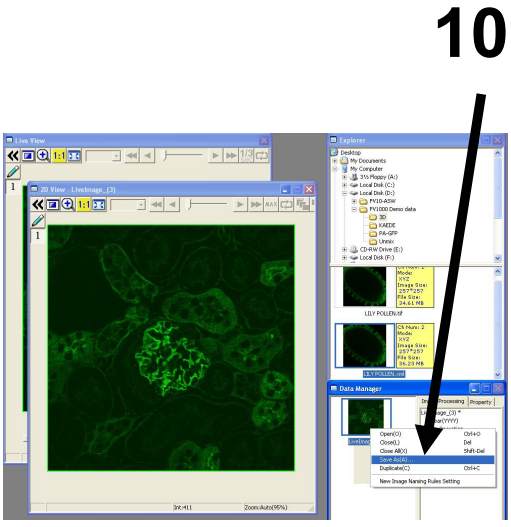


① Sensitivity of detector adjustment (HV)
② Confocal aperture adjustment (CA)
③ Laser output adjustment (Laser)

Adjustment method (Example: HV)
When any place on slider is clicked, the HV can be jumped UP or DOWN to the point where it is clicked. For fine tuning, click ▲ or use mouse wheel.







7. Select AutoHV and select ScanSpeed.

   * The slower the speed set, the more the noise only can be reduced by keeping current brightness.
   (In addition, Kalman integration is available as a separate method to remove noise.
   For further details, see Annex 2.)

8. Click XY button to acquire an image.
9. When acquisition is completed, “2D View-(File Name)” will appear on title bar of the image acquired.
10. Image save:
    Click mouse right button over Image displayed on DataManager and then, select SaveAs.

    (Save as Type “oib” or “oif” is the dedicated file format for FV10-ASW software.)

■Memo ■
Dedicated file format for FV10-ASW

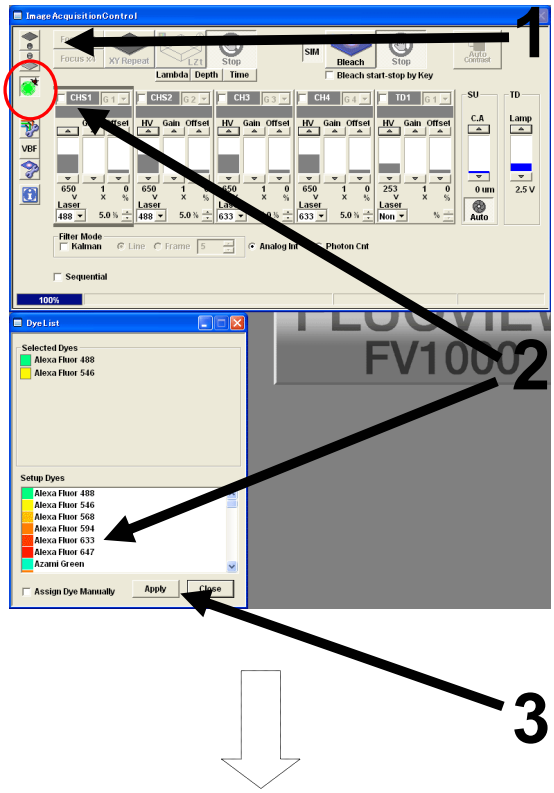
OIF type:
Folder that contains images (16bit TIFF) and attached file are created. Unless these two exist, the file cannot be opened.
OIB type:
File that contains a plural number of OIF files. It is convenience when files are moved.

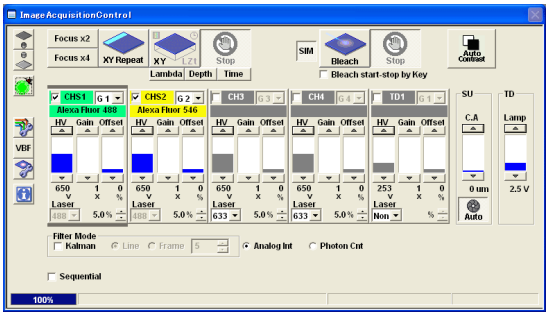
3.9.Image acquisition (Double dyeing color XY)

■■ 1 slice of image acquisition (XY plane) (Fluorescent image only) ■■

Example: Green fluorescence (Alexa488) + Red fluorescence (Alexa546)

Double dyeing simultaneous scan version





Window after DyeApply clicked

1. Click of FV10-ASW software and set it to “not pressed” state and close shutter of fluorescent lamp.
   Click and set it to “not pressed” state and close shutter of halogen lamp.

2. Click DyeList button and select fluorescent reagent (Alexa488, Alexa546) from DyeList panel and double-click it.

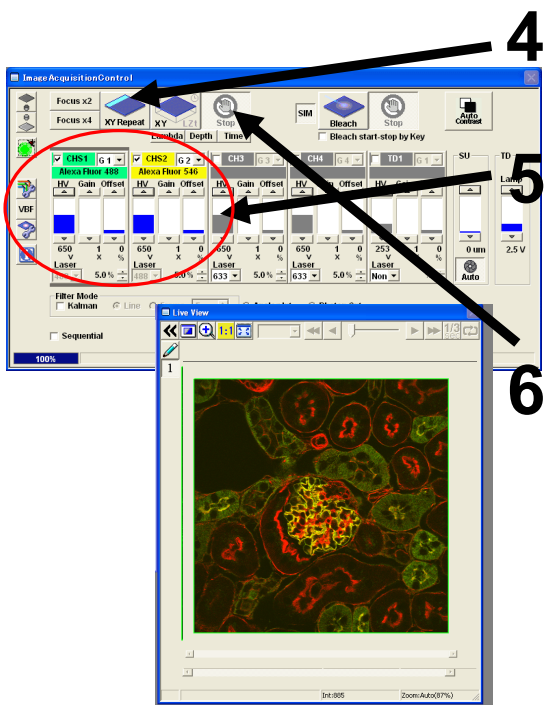
   * When reselecting, double-click the fluorescent reagent in AssignDyes one time to delete it and then, do procedure 2.

3. Click Apply button.

   (DyeList panel can be closed with Close button.)

- In case of Spectral type -
When this operation is done, the fluorescent wavelength suit to Fluorescent Dye selected is defined.

Moreover, the fluorescent wavelength is manually changeable.
For further details, see Annex 3.



4. Click XY Repeat button to perform scanning.

5. Adjust image of Green (AlexaFluor488) and Red (AlexaFluor546). (See below regarding outline of image adjustment. For further details, see Annex 1.)

6. Click STOP button to stop scanning. (Ref: ■Memo■)

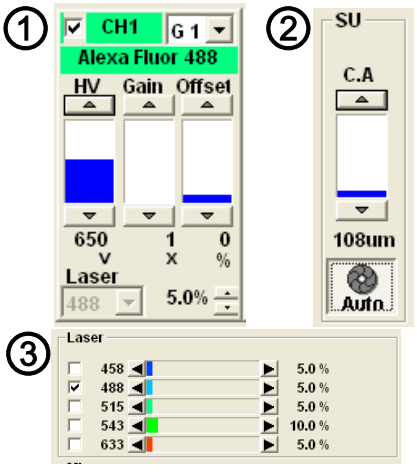
■ Memo ■
Scan buttons

: Consecutive scan

: Scan stop

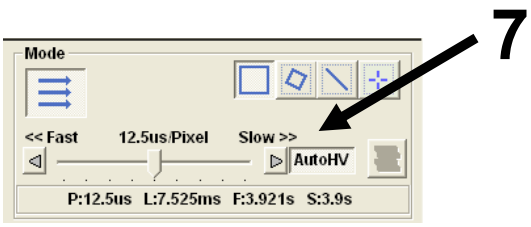
: Rough scan
(Lines skipped and scanned)

Outline of image adjustment

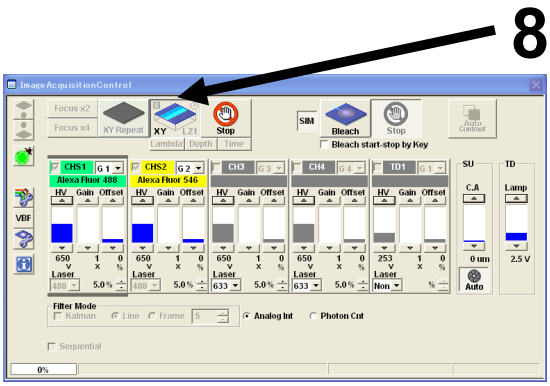


① Sensitivity adjustment of detector (HV)
② Confocal aperture adjustment (CA)
③ Laser output adjustment (Laser)

Adjustment method (Example: HV)
When any place on slider is clicked, the HV can be jumped UP or DOWN to the point where it is clicked. For fine tuning, click ▲ or use mouse wheel.



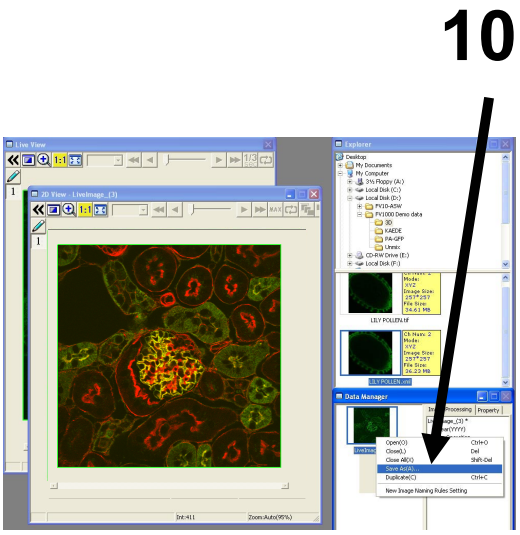
7. Select **AutoHV** and select **ScanSpeed**.
   * The slower the speed set, the more the noise only can be reduced by keeping current brightness.
   (In addition, Kalman integration is available as a separate method to remove noise. For further details, see Annex 2.)



8. Click XY button to acquire an image.

9. When acquisition is completed, "2D View-(File Name)" will appear on title bar of the image acquired.

10. Image save:
    Click mouse right button over Image displayed on DataManager and then, select SaveAs.
    (Save as Type "oib" or "oif" is the dedicated file format for FV10-ASW software.)



■Memo ■
Dedicated file format for FV10-ASW

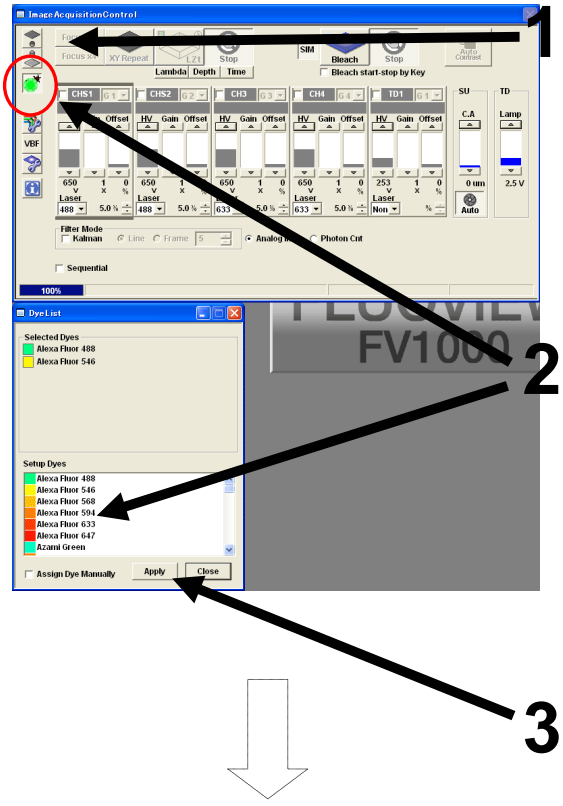
OIF type:
Folder that contains images (16bit TIFF) and attached file are created. Unless these two exist, the file cannot be opened.
OIB type:
File that contains a plural number of OIF files. It is convenience when files are moved.

3.10.Image acquisition (Double dyeing color XY)

■■ 1 slice of image acquisition (XY plane) (Fluorescent image only) ■■

Example: Green fluorescence (Alexa488) + Red fluorescence (Alexa546)

Double Dyeing Sequential Scan Version (Line Sequential introduced)



1. Click of FV10-ASW software and set it to “not pressed” state and close shutter of fluorescent lamp.

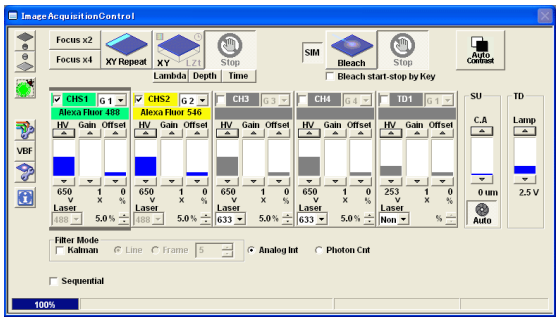
   Click and set it to “not pressed” state and close shutter of halogen lamp.

2. Click DyeList button and select fluorescent reagent (Alexa488, Alexa546) from DyeList panel and double-click it.

   * When reselecting, double-click the fluorescent reagent in AssignDyes one time to delete it and then, do procedure 2.

3. Click Apply button.

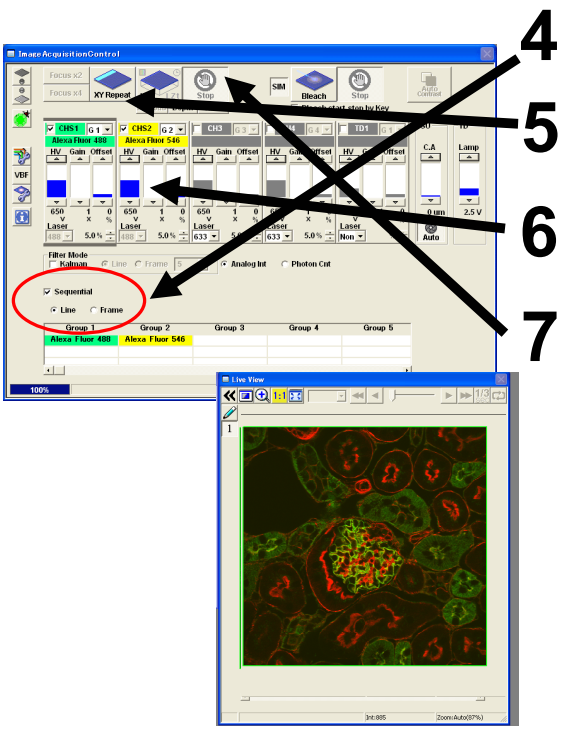
   (DyeList panel can be closed with Close button.)



Window after DyeApply clicked

- In case of Spectral type -
When this operation is done, the fluorescent wavelength suit to Fluorescent Dye selected is defined.

Moreover, the fluorescent wavelength is manually changeable.
For further details, see Annex 3.



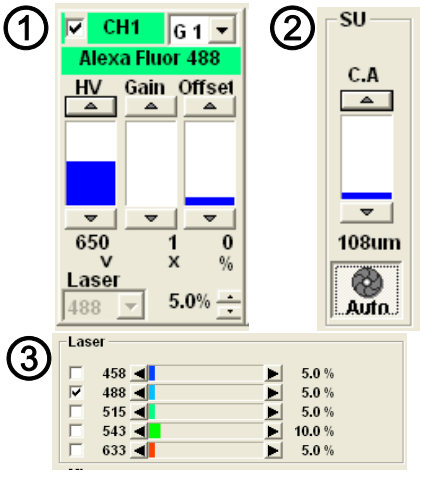
4. Check Sequential and select Line.

5 Click XY Repeat button to perform scanning.

6. Adjust image of Green (AlexaFluor488) and Red (AlexaFluor546). (See below regarding image adjustment outline. For further details, see Annex 1.)

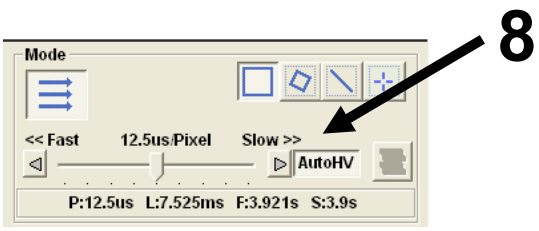
7. Click STOP button to stop scanning

Outline of image adjustment

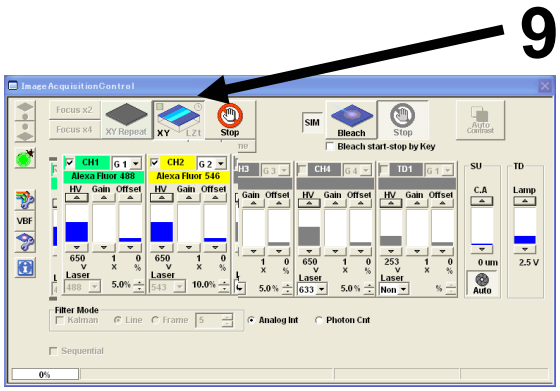


① Sensitivity adjustment of detector (HV)
② Confocal aperture adjustment (CA)
③ Laser output adjustment (Laser)

Adjustment method (Example: HV)
When any place on slider is clicked, the HV can be jumped UP or DOWN to the point where it is clicked. For fine tuning, click ▲ or use mouse wheel.



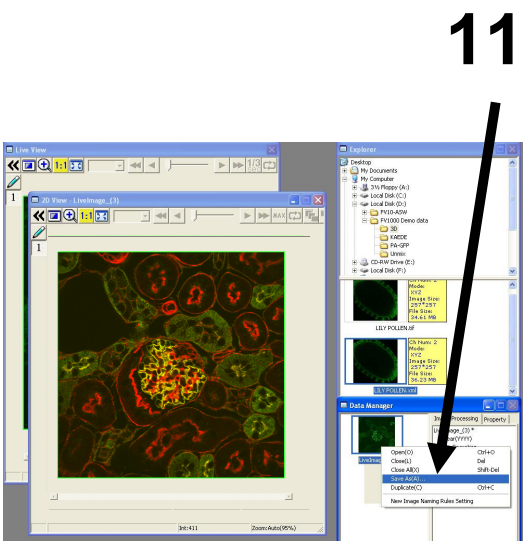
8. Select **AutoHV** and select **ScanSpeed**.
   * The slower the speed set, the more the noise only can be reduced by keeping current brightness.
   (In addition, Kalman integration is available as a separate method to remove noise. For further details, see Annex 2.)



9. Click XY button to acquire an image.

10. When acquisition is completed, "2D View-(File Name)" will appear on title bar of the image acquired.

11. Image save:
    Click mouse right button over Image displayed on DataManager and then, select SaveAs.
    (Save as Type "oib" or "oif" is the dedicated file format for FV10-ASW software.)



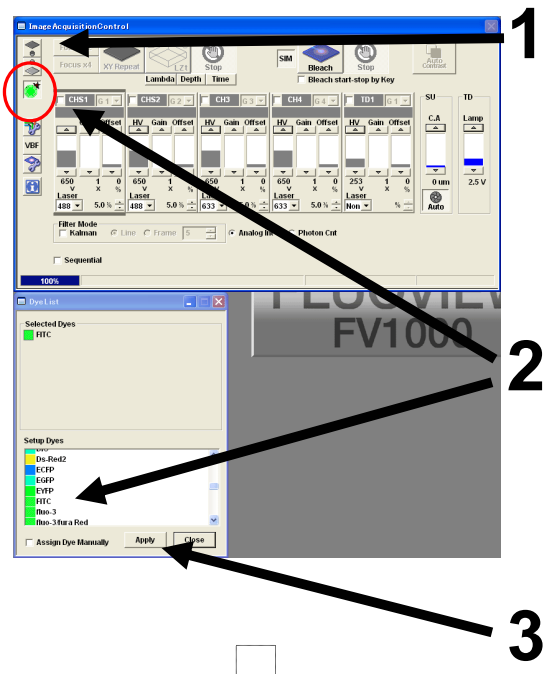
■Memo ■
Dedicated file format for FV10-ASW

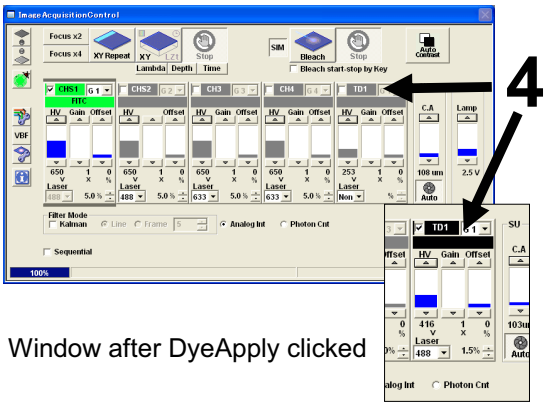
OIF type:
Folder that contains images (16bit TIFF) and attached file are created. Unless these two exist, the file cannot be opened.
OIB type:
File that contains a plural number of OIF files. It is convenience when files are moved.

3.11.Image acquisition (Single dyeing color + DIC XY)

■■ 1-slice Image (XY plane) acquisition (Fluorescent Image + Differential Interference)■■

Example: Green fluorescence (FITC) + Differential Interference





Window after DyeApply clicked

1. Click of FV10-ASW software and set it to “not pressed” state and close shutter of fluorescent lamp.

   Click and set it to “not pressed” state and close shutter of halogen lamp.

2. Click DyeList button and select fluorescent reagent (FITC) to be observed from DyeList panel and double-click it.

   * When reselecting, double-click the fluorescent dye in AssignDyes one time to clear it and then, perform procedure 2.

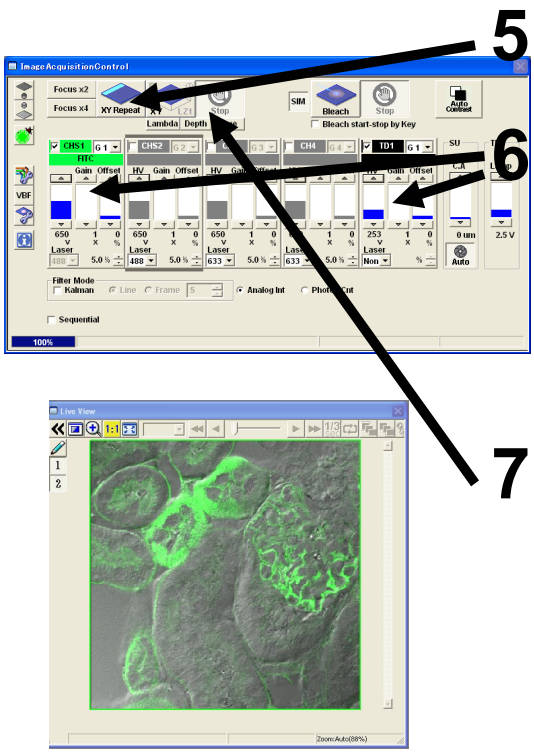
3. Click Apply button.

   (DyeList panel can be closed with Close button.)

4. Check TD1.

- In case of Spectral type -
When this operation is done, the fluorescent wavelength suit to Fluorescent Dye selected is defined.

Moreover, the fluorescent wavelength is manually changeable.
For further details, see Annex 3.



5. Click XY Repeat button to perform scanning.

6. Adjust Green (FITC) image and differential interference image. (See below regarding image adjustment outline. For further details, see Annex 1.)

7. Click STOP button to stop scanning. (Ref: ■Memo■)

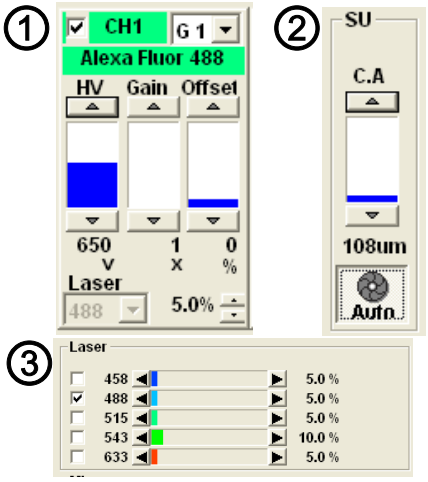
■ Memo ■
Scan buttons

: Consecutive scan

: Scan stop

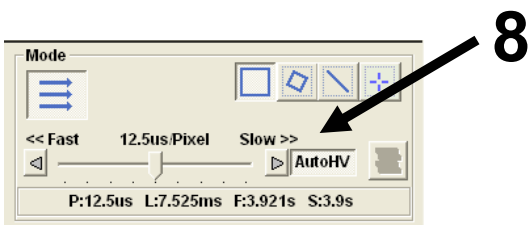
: Rough scan
(Line skipped and scanned)

Outline of image adjustment

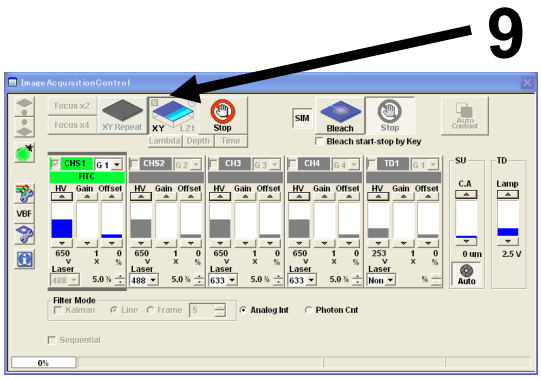


① Sensitivity adjustment of detector (HV)
② Confocal aperture adjustment (CA)
③ Laser output adjustment (Laser)

Adjustment method (Example: HV)
When any place on slider is clicked, the HV can be jumped UP or DOWN to the point where it is clicked. For fine tuning, click or use mouse wheel.

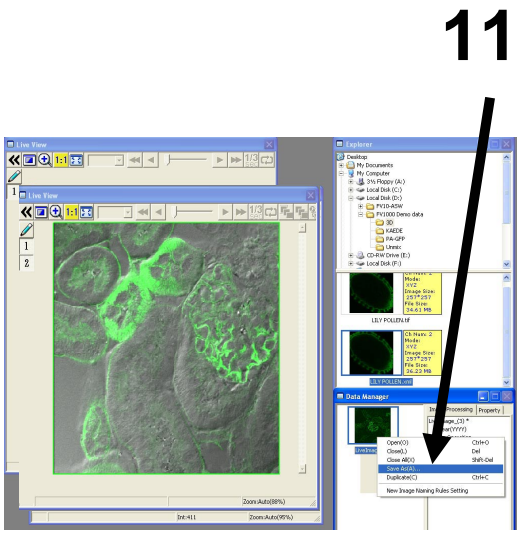


8. Select **AutoHV** and select **ScanSpeed**.
   * The slower the speed set, the more the noise only can be reduced by keeping current brightness.
   (In addition, Kalman integration is available as a separate method to remove noise. For further details, see Annex 2.)



9. Click XY button to acquire an image.

10. When acquisition is completed, "2D View-)File Name)" will appear on title bar of the image acquired.



11. Image save:
    Click mouse right button over Image displayed on **DataManager** and then, select SaveAs.
    (Save as Type "oib" or "oif" is the dedicated file format for FV10-ASW software.)

■Memo ■
Dedicated file format for FV10-ASW

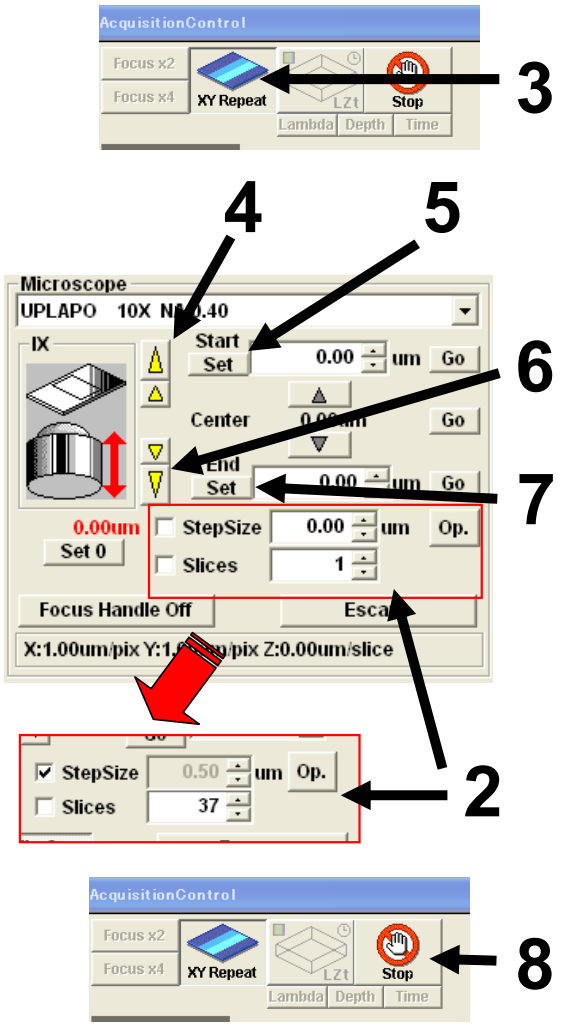
OIF type:
Folder that contains images (16bit TIFF) and attached file are created. Unless these two exist, the file cannot be opened.
OIB type:
File that contains a plural number of OIF files. It is convenience when files are moved.

3.12.Image acquisition (Double dyeing color XYZ)

■■ Consecutive Cross-section Image (XYZ) Acquisition (Fluorescent Image only)■■

Example:Green fluorescence (Alexa488)+Red fluorescence (Alexa546) Double Dyeing

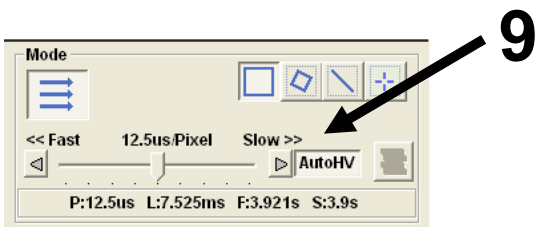
Cross-section image acquired with Line Sequential scanning



1. Perform procedures 1 through 7 described in page 26 and 27.

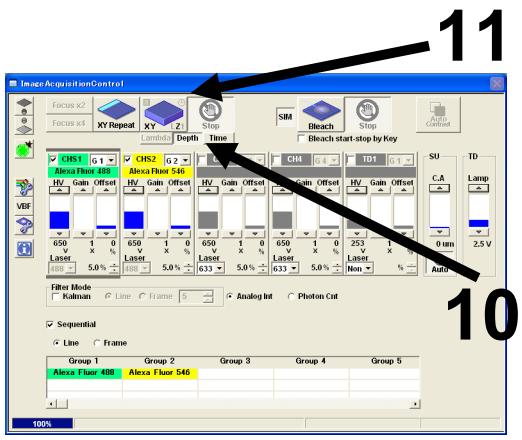
Upper and lower limit of consecutive cross-section image is determined here.

2. Enter StepSize and tick the box.
3. Click XY Repeat button to perform scanning.
4. Click or to bring the object in out of focus.
5. When sample upper limit is displayed on the sample, click Set button.
6. Click or to bring the object in out of focus.
7. When sampler lower limit is displayed on the sample, click Set button.
8. Click Stop button and stop scanning.



9. Select AutoHV and select ScanSpeed.

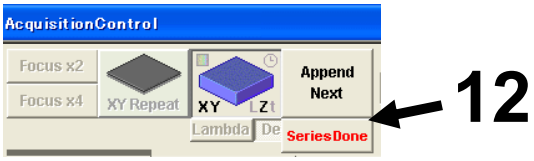
∗ The slower the speed set, the more the noise only can be reduced by keeping current brightness.
(In addition, Kalman integration is available as a separate method to remove noise.
For further details, see Annex 2.)



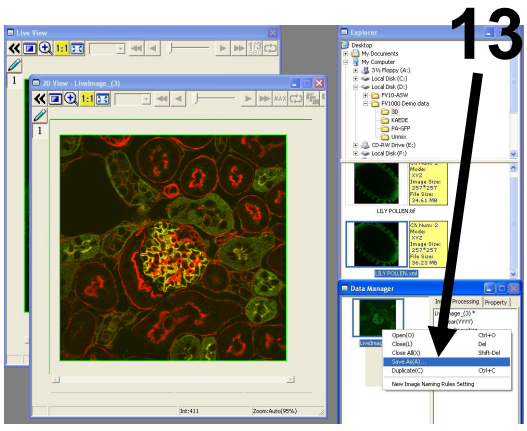
10. Select Depth.

11. Click XYZ button to acquire an image

12. Click SeriesDone so that, on Title bar of the image acquired, 2D View-(file name) will appear.



13. Image save:
Click mouse right button over ImageIcon displayed on DataManager and then, select SaveAs.
(Save as Type “oib” or “oif” is the dedicated file format for FV10-ASW software.)



■Memo ■
Dedicated file format for FV10-ASW

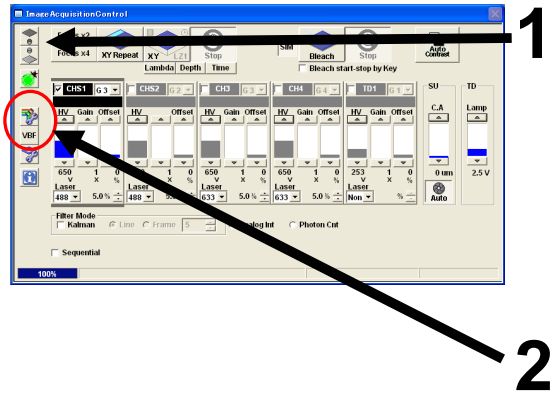
OIF type:
Folder that contains images (16bit TIFF) and attached file are created. Unless these two exist, the file cannot be opened.
OIB type:
File that contains a plural number of OIF files. It is convenience when files are moved.

3.13.Image acquisition (Spectral XYL) (Spectral type)

■■ Spectral Image (XYL) Acquisition ■■

Example: Green fluorescence (AlexaFluor488) + Green fluorescence (YOYO-1)

Double dyeing



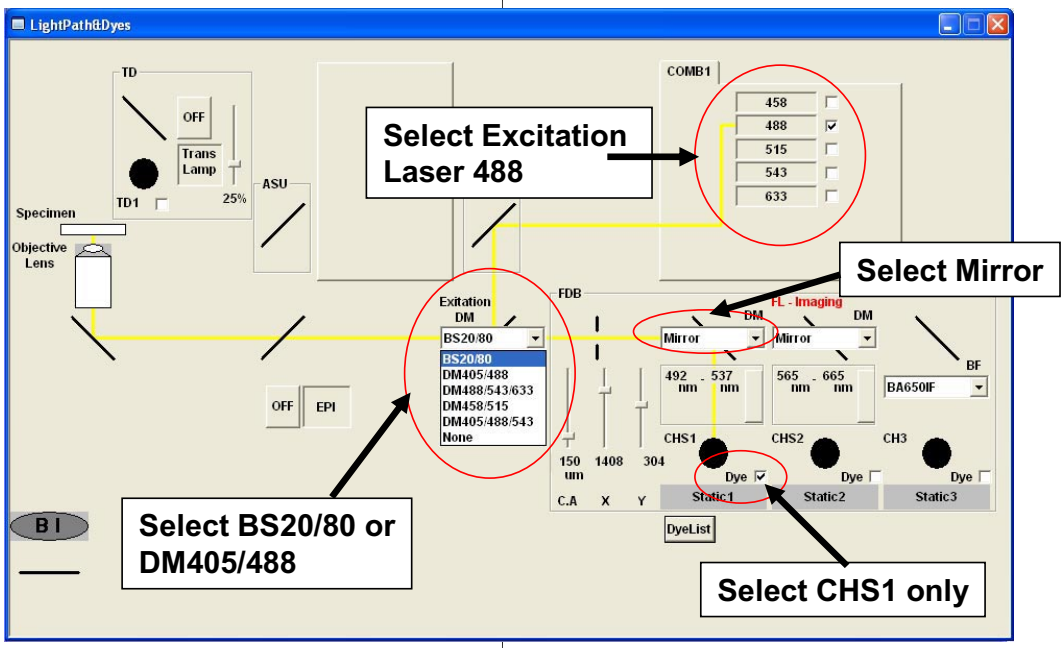
1. Click of FV10-ASW software and set it to “not pressed” state and close shutter of fluorescent lamp.

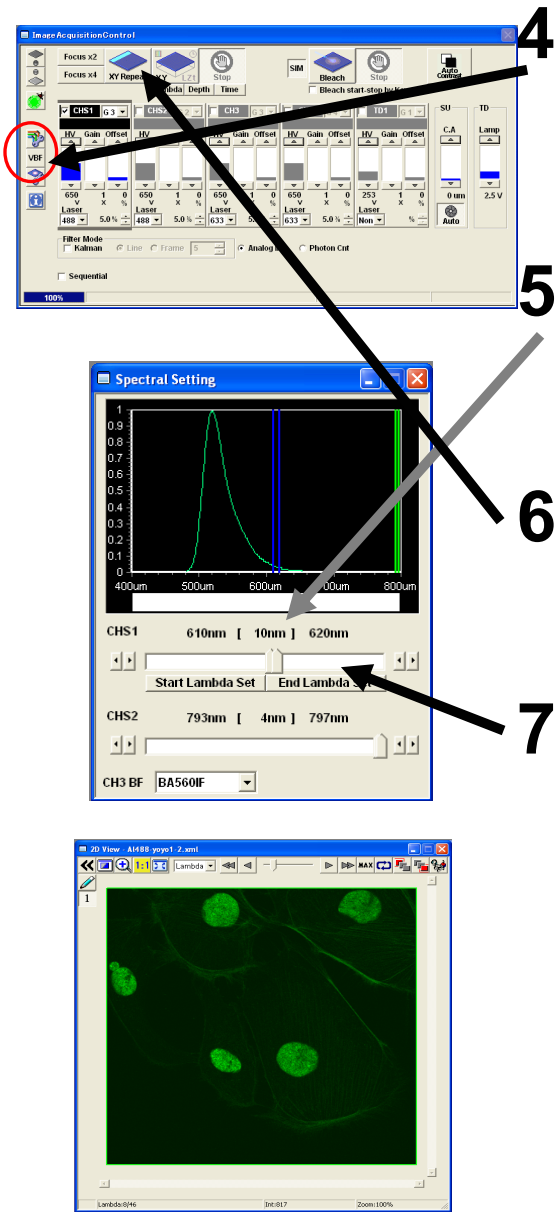
   Click and set it to “not pressed” state and close shutter of halogen lamp.

2. Click and display light path diagram.

3. Set as shown below.

3





4. Click VBF button and display SpectralSetting window.

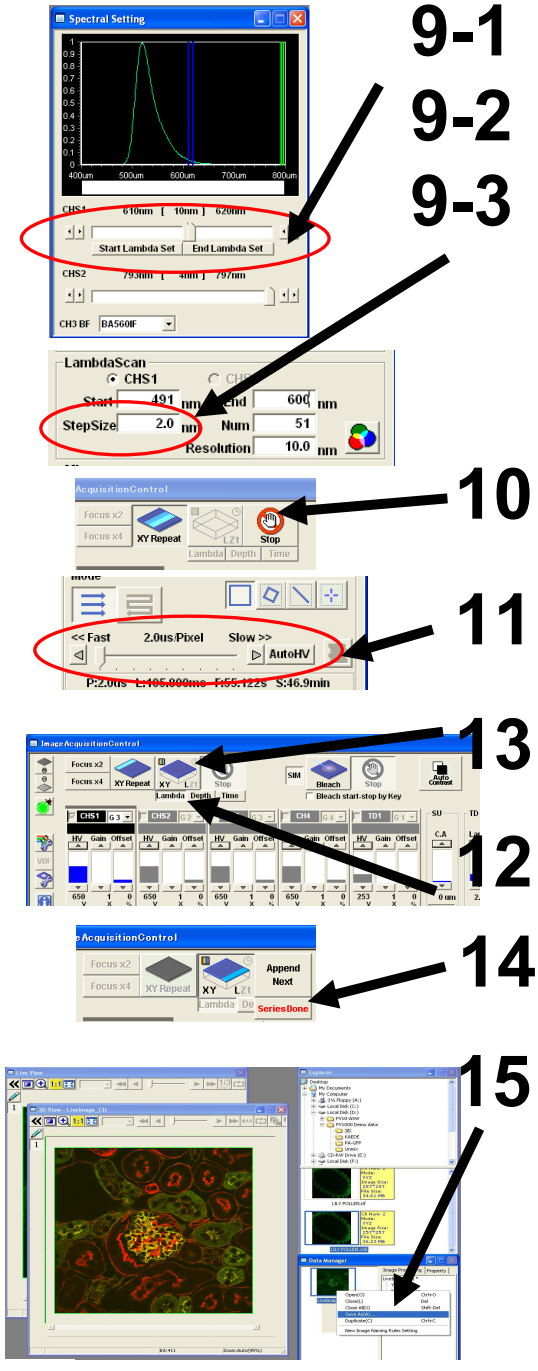
5. Set slit width of CHS1 to 10nm (as example).
   (To change slit width, see Annex 3.)

6. Click XY Repeat button to perform scanning.

7. Viewing the image, move slit position to the brightest position.
   (To change slit position, see Annex 3.)

   Note: Leave slit width to 10nm “as is” and move position only.

8. Adjust the image.
   (Regarding image adjustment, see Annex 1.)



9. Setting range/step of wavelength to be acquired.

9-1 : Move slit to wavelength start and click “Start LambdaSet”.
9-2 : Move slit to wavelength end and click “End LambdaSet”.
9-3 : Enter Step Size.

10. Stop scanning.

11. Select AutoHV and select ScanSpeed.

∗ The slower the speed set, the more the noise only can be reduced by keeping current brightness.
(In addition, Kalman integration is available as a separate method to remove noise.
For further details, see Annex 2.)

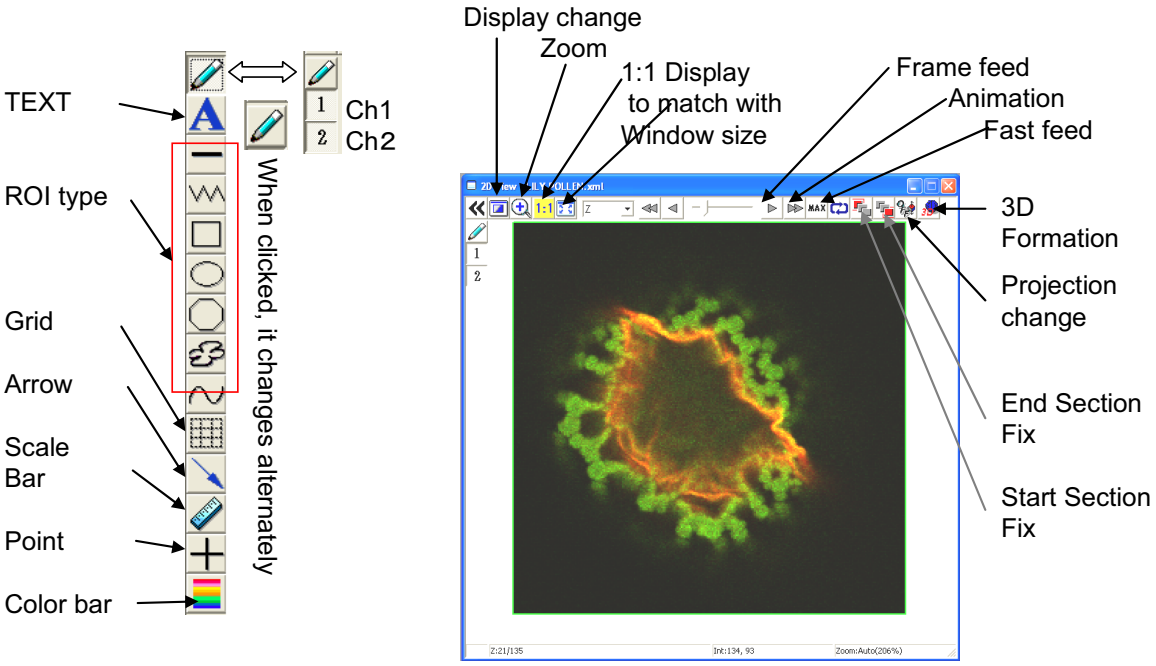
12. Select Lambda.

13. Click XYL button to acquire an image.

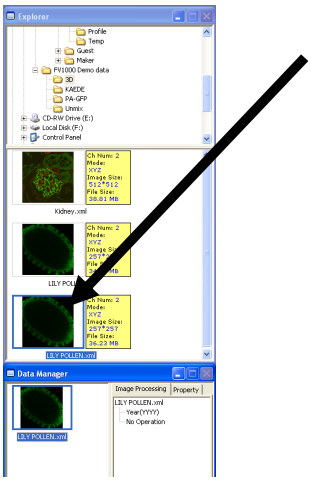
14. Click SeriesDone so that, on Title bar of the image acquired, 2DView-(file name) will appear.

15. Image save:
Click mouse right button over ImageIcon displayed on DataManager and then, select SaveAs.
(Save as Type “oib” or “oif” is the dedicated file format for FV10-ASW software.)

3.14. 2D Operation Panel Outline

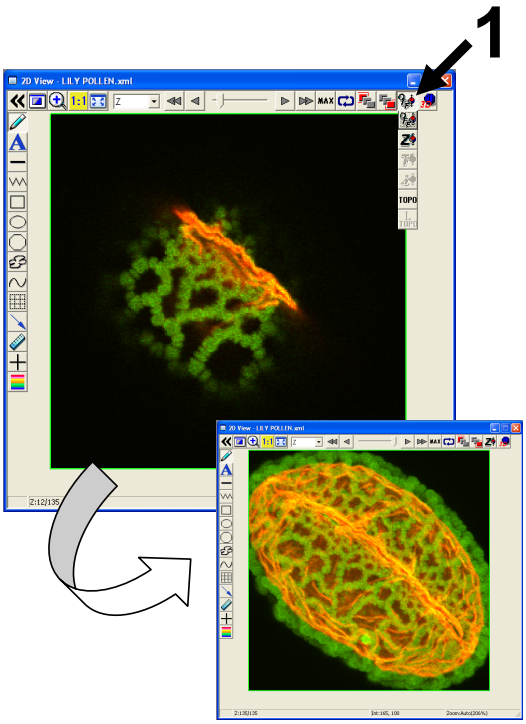


3.15.File open

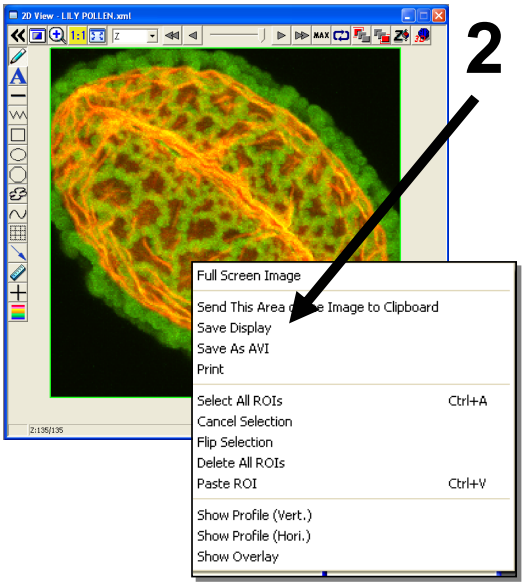


1. Double-click a file that should be opened from Explorer.

3.16. Display of XYZ Image (Cross-section image overlap)

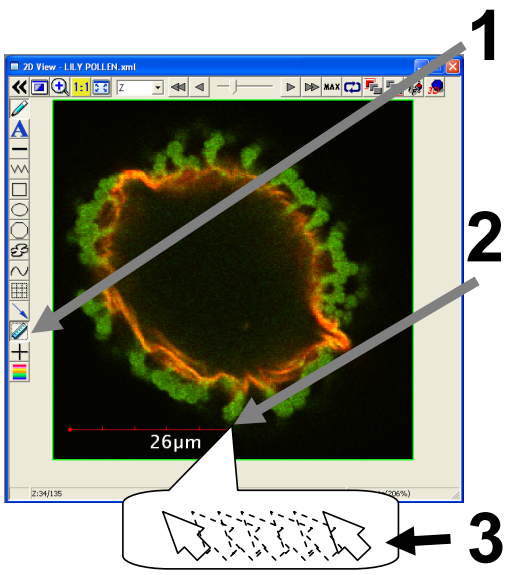


1. Click and select .



2. In case that this image is saved, click mouse right button over the image and select Save Display and save it with a name assigned.

3.17.Putting Scale bar

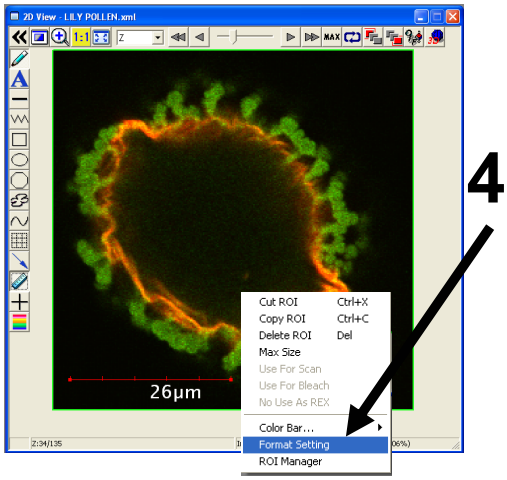


1. Click button.

2. While left mouse button is clicked over the image, drag the mouse and release the button at proper point.

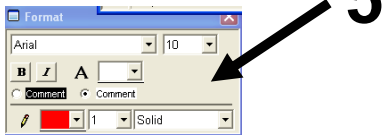
Size change

3. While right or left handle is clicked, move the mouse, left or right.



Change of character size, color and style, etc.

4. After selecting ScaleBar, click mouse right button over ScaleBar and select “FormatSetting”.



5. In this Window, change portions that must be changed.

3.18. 3D Display

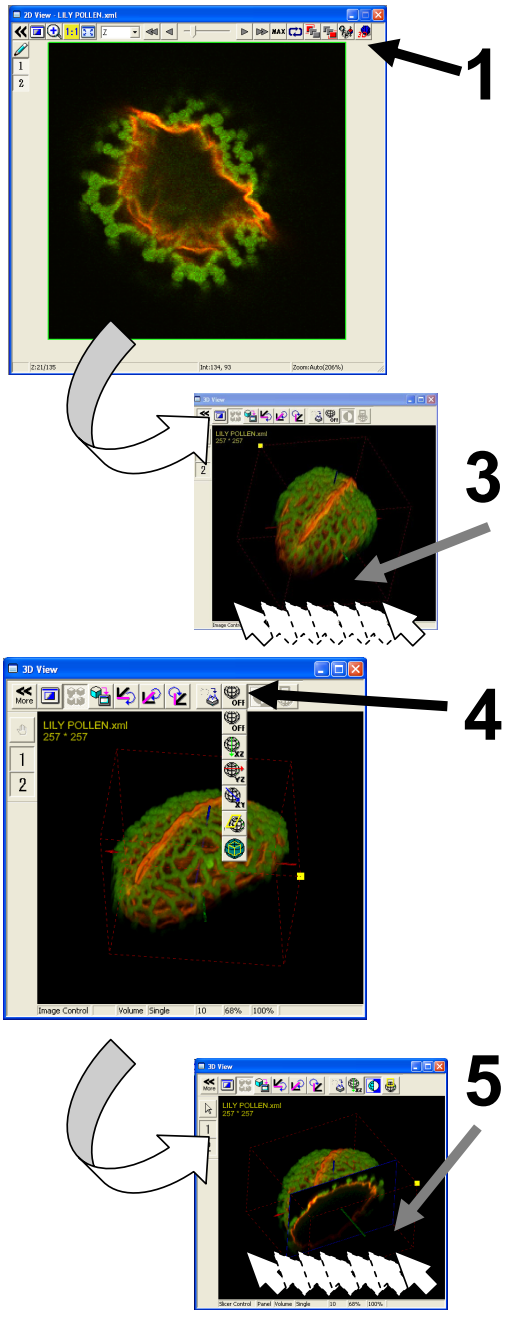


Image is observed from arbitrarily selected angle.

1. Click button on 2D View-(file name) image.

2. [3D-OLYMPUS FLUOVIEW] window starts up and 3D View will be built up.

3. Dragging mouse over the image, observe the image from arbitrarily selected angle.

→ To save this image, see procedure 6 in next page.

Cross-section image at optional angle is observed.

4. Click and select .

5. Dragging mouse over the image, move, left or right, to see cross-section at optional angle.

→ To save this image, see procedure 6 in next page.

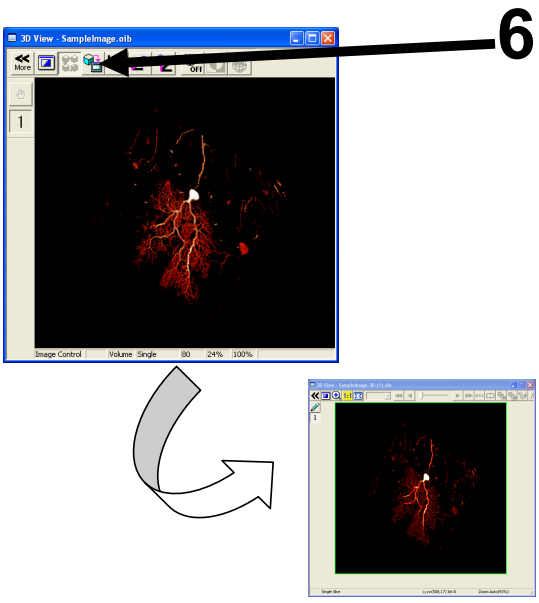
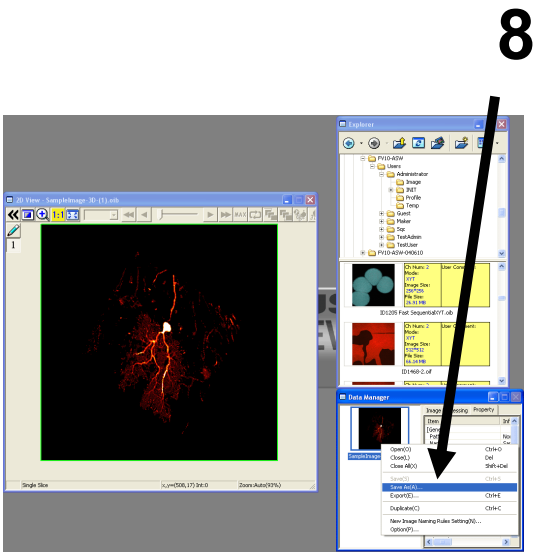


Image in procedure 3 or 5 is saved.

6. Click button.

7. 2D View-(file name) image appears.



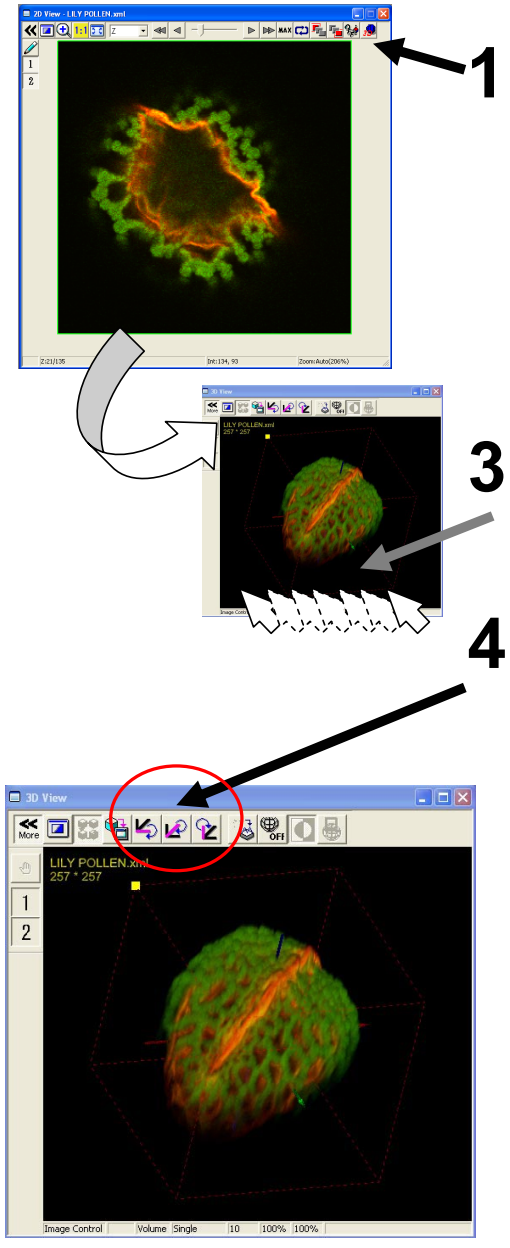
8. Image save:
   Click mouse right button over Image displayed on DataManager and then, select SaveAs.

   (Save as Type “oib” or “oif” is the dedicated file format for FV10-ASW software.)

■Memo ■
Dedicated file format for FV10-ASW

OIF type:
Folder that contains images (16bit TIFF) and attached file are created. Unless these two exist, the file cannot be opened.
OIB type:
File that contains a plural number of OIF files. It is convenience when files are moved.

3.19.Rotation of cubic image



1. Click button on 2D View-(file name) image.

2. [3D-OLYMPUS FLUOVIEW] window starts up and 3D View will be built up.

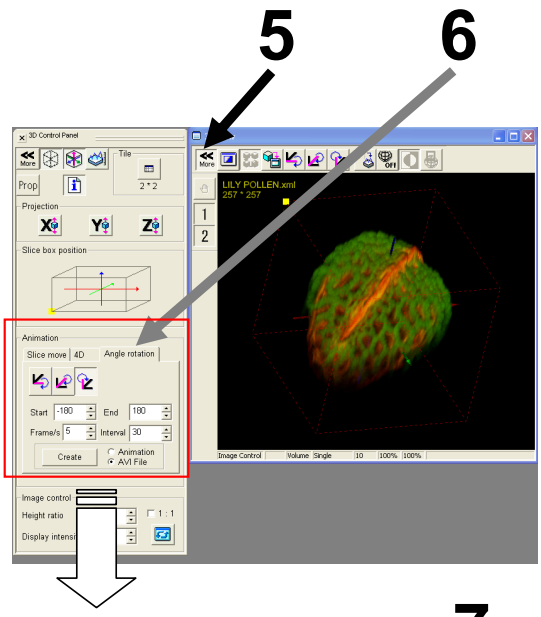
3. Dragging mouse over the image, observe the image from arbitrarily selected angle.

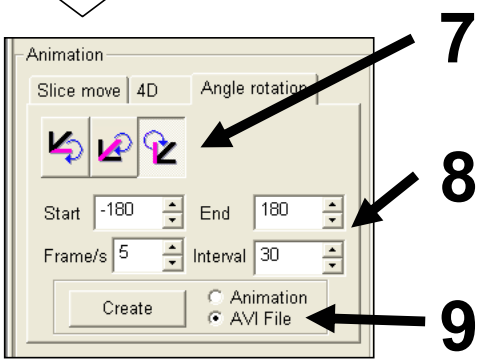
Simplified animation

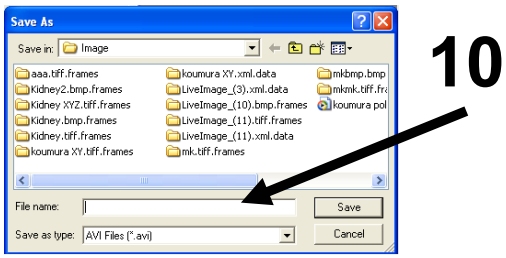
4. When button is clicked with long mode, the image turns with X-axis as rotation center. When clicked again, the turn would stop.

When button is clicked with long press mode, the image turns with Y-axis as rotation center. When clicked again, the turn would stop.

When button is clicked with long press mode, the image turns with Z-axis as rotation center. When clicked again, the turn would stop.

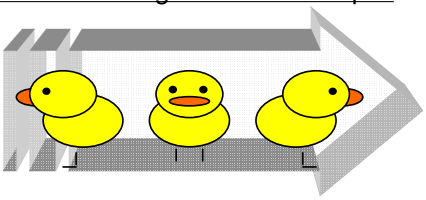






When a rotation file is saved as movie, 3D formation must be executed as follows.

Let's turn an image 180° as example.



5. Click

button.

6. Double-click Angle Rotation tab.

7. Select rotation angle axis.

8. Enter rotation angle.

Start＝From what degree / End＝To what degree
Frame/s＝Rotation speed / Interval＝per what degree

9. Select AVI File and click Create.

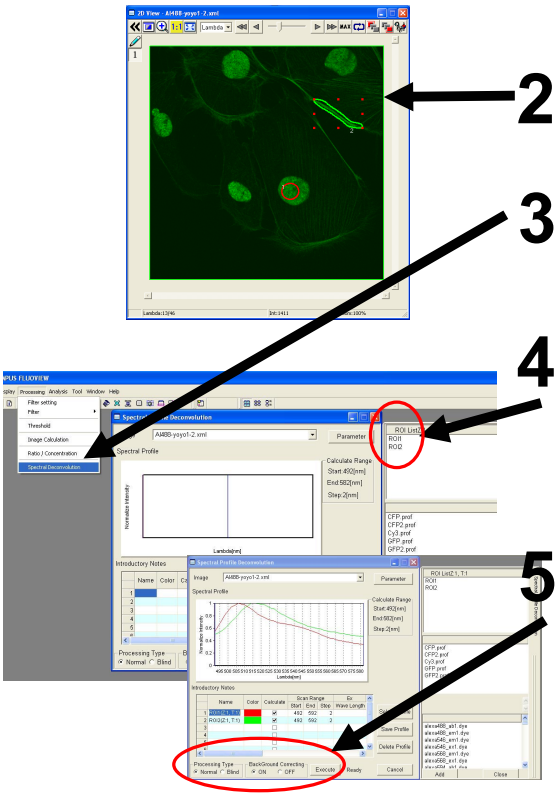
10. Enter file name and click Save.

3.20.Fluorescence separation - unmixing (Spectral type)

Part 1: In case that Dye place for each fluorescence is known

Fluorescent spectrum of each fluorescent dye are extracted from XYL image where a plural number of fluorescent dyes having fluorescent spectrum similar to each other exist and; with the said fluorescent spectrum as reference, a method is presented here to acquire a fluorescence separated image.

Example: Green fluorescence (AlexaFluor488) + Green fluorescence (YOYO-1)



1. Open XYL image file of AlexaFluor488 + YOYO1 of double dyeing.
2. Surround AlexaFluor488 and YOYO1 region only with ROI.
3. Select SpectralDeconvolution from Processing on menu bar.
4. Double-click ROI1 and ROI2 respectively.
5. Verify that Processing type is set to “Normal” and BackGround Correcting is turned “ON” and then, click NewImage.
6. Image of fluorescent separation can be acquired.

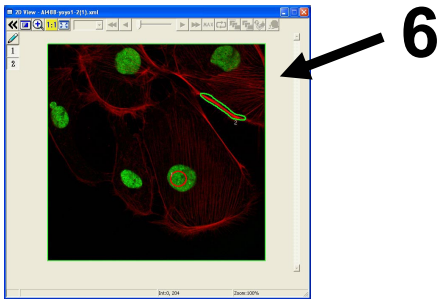
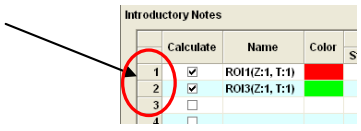


Image after fluorescence separating (Unmixing)

After fluorescence separation (Unmixing), the assignment of image to Ch is indicated.

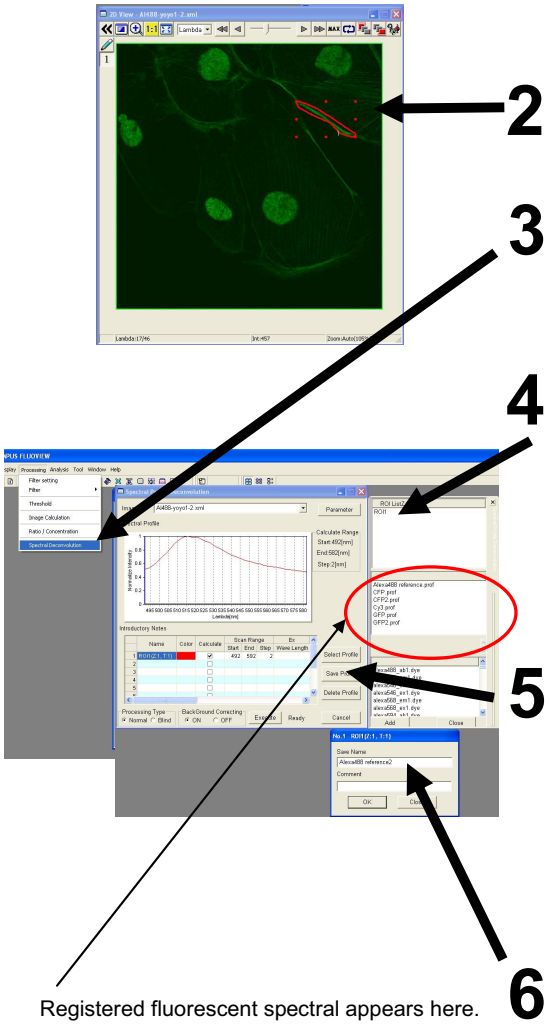


3.21.Fluorescence separation - unmixing (Spectral type)

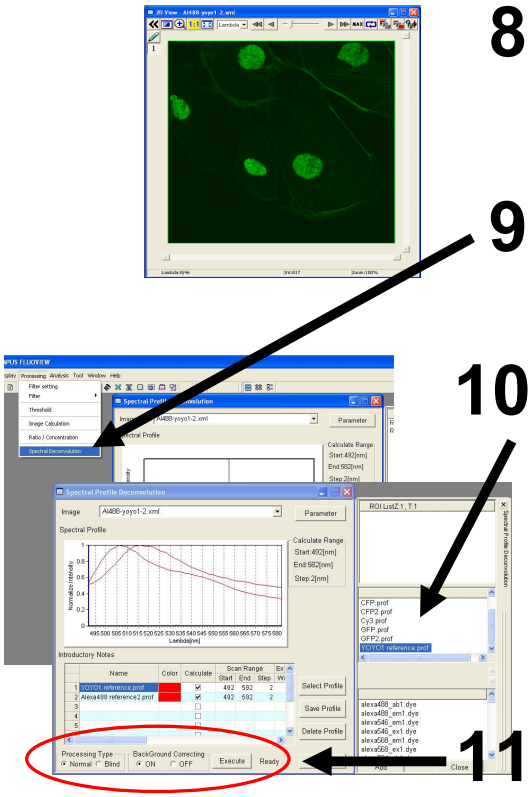
Part 2: In case that control sample is used

Method to acquire a fluorescence separated image with reference to fluorescent spectrum. The said spectrum are extracted from one kind of fluorescent dye on XYL image.

Example: Green fluorescence (AlexaFluor488) + Green fluorescence (YOYO-1) Double Dyeing



1. Open XYL image file of control sample (the sample dyed with AlexaFluor488 only).
2. Surround AlexaFluor488 region with ROI.
3. Select SpectralDeconvolution from Processing on menu bar.
4. Double-click ROI1.
5. Click SaveProfile.
6. Enter name to be saved and click OK and the fluorescent spectral of AlexaFluor488 is registered to data base.
7. Perform the same operation, 1 to 6, for YOYO1.



8. Open XYL image file of AlexaFluor488 + YOYO1 of double dyeing.

9. Select SpectralDeconvolution from Processing on menu bar.

10. Double-click AlexaFluor488 and YOYO1 (previously registered) from data base of fluorescent spectrum.

11. Verify that Processing type is set to “Normal” and BackGround Correcting is turned “ON” and then, click NewImage.

12. Image of fluorescence separated can be acquired.

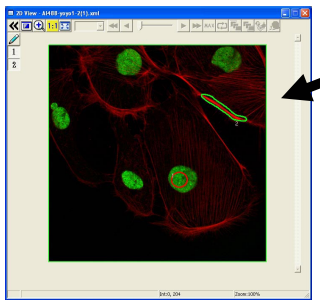
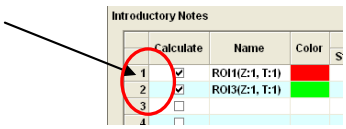


Image after fluorescence separating (Unmixing)

After fluorescence separation (Unmixing), the assignment of image to Ch is indicated.

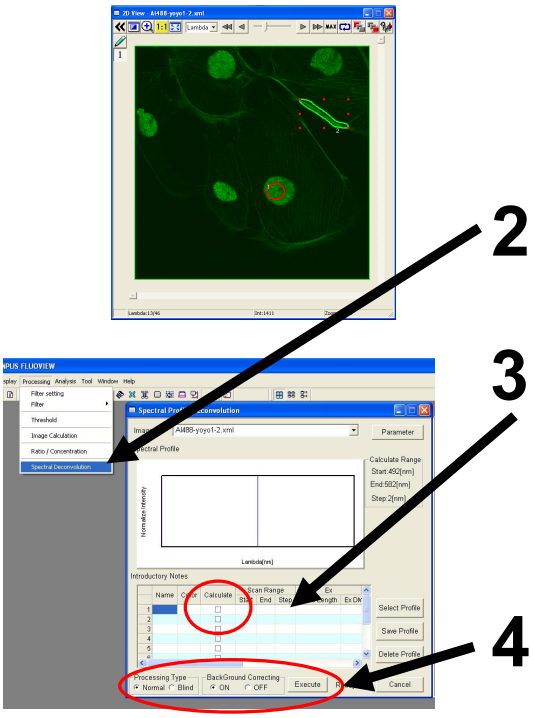


3.22.Fluorescence separation - unmixing (Spectral type)

Part 3: In case that number of fluorescent dye kinds only is known (Blind Unmixing)

Method to acquire a fluorescence separated image with a little information such as number of fluorescent dye kinds only known as a hint from XYL image where a plural number of fluorescent dyes having fluorescent spectrum similar to each other exist.

Example: Sample having 2 kinds of unknown fluorescent dyes



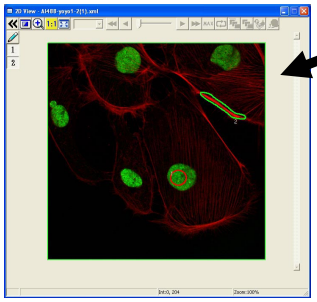
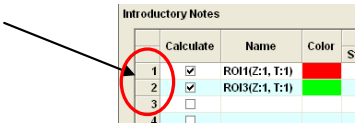


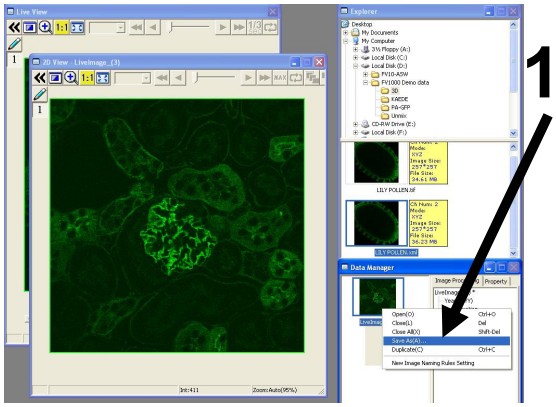
Image after fluorescence separating (Unmixing)

1. Open XYL image file of sample that has 2 kinds of unknown fluorescent dyes.
2. Select SpectralDeconvolution from Processing on menu bar.
3. Put check marks at 2 places for Calculate check box. (When 3 kinds of fluorescent Dyes exist, click 3 places to put check marks.)
4. Verify that Processing type is set to “Blind” BackGround Correcting is turned “ON” and then, click NewImage.
5. Image of fluorescence separated can be acquired.

After fluorescence separation (Unmixing), the assignment of image to Ch is indicated.



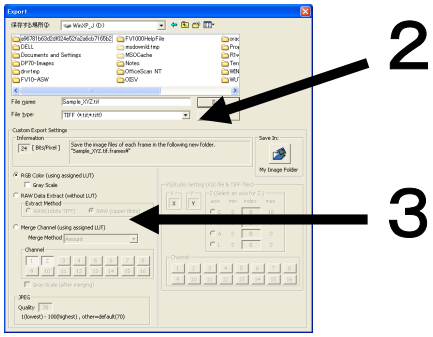
3.23.Image save



Each channel for XY or XYZ Image is converted to TIFF.

1. Click mouse right button over ImageIcon displayed on DataManager and select Export.
2. Set Save as Type to TIFF and save.

* Other type, BMP/JPEG/PNG can be selected.



Merge image of XY or XYZ Image is converted to TIFF.

1. Click mouse right button over ImageIcon displayed on DataManager and select Export.
2. Set Save as Type to TIFF.
3. Check MergeChannel and save.

* Other type, BMP/JPEG/PNG can be selected.

****

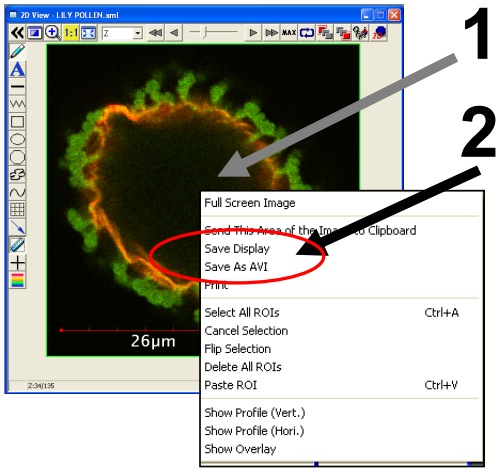


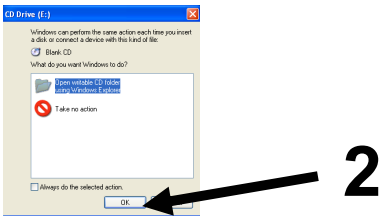
Image with Scale bar is converted to BMP.

1. Click mouse right button over image.
2. Select Save Display and save the image with a name assigned.

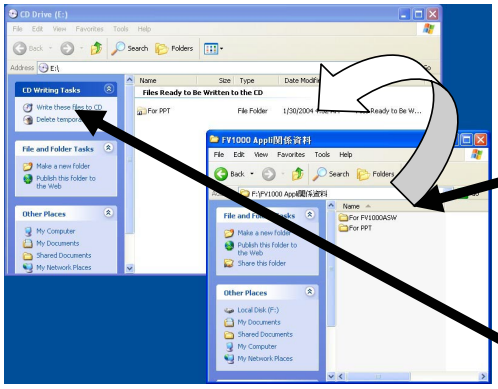
Movie is converted to AVI.

1. Click mouse right button over image.
2. Select Save as AVI and save the image with a name assigned.

3.24.Save to CD-R



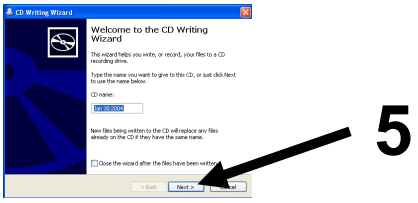
1. Insert CD-R media.



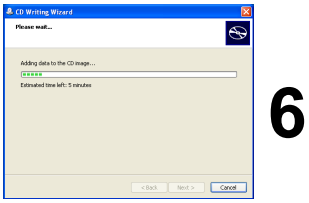
2. Click OK.

3. Select a file and move it to Window for CD-R with drag-and-drop technique.

4. Click Write these files to CD.



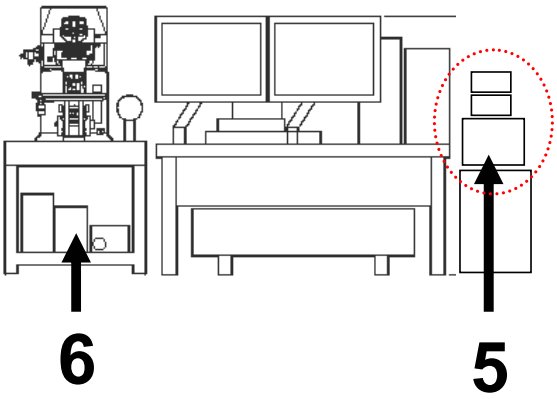
5. Click Next.



6. Write will start.

7. Click Finish so that the save to CD-R is completed.

3.25.System shut down



1. Shut donw FV10-ASW software with File/Exit.

2. Shut down WindowsXP.
① Select Start - Shut Down.
② Select “Shutdonwn” on ShutDownWindow and click OK.

3. Turn FV10-PSU to OFF.

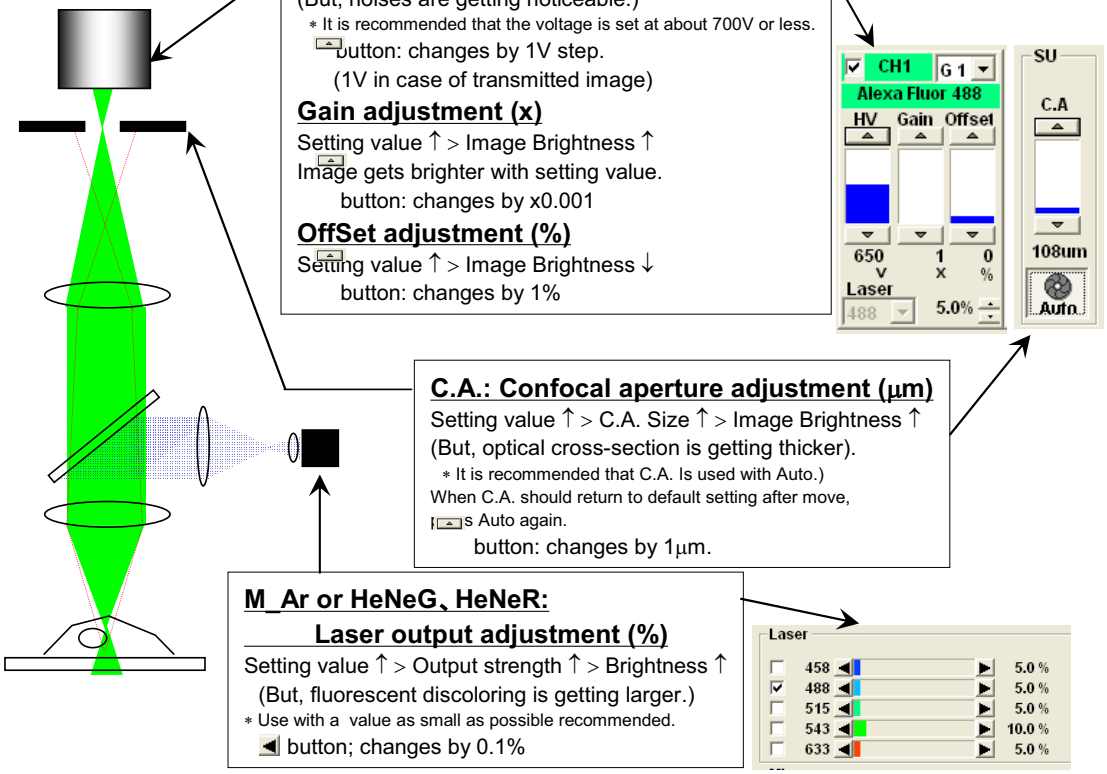
4. Turn BX-UCB or IX2-UCB to OFF.

5. Turn laser to OFF.
(Return key switch to OFF position)
5-1. Multi Ar
（458nm･488nm･514nm） OFF
5-2. HeNe（G）(543nm） OFF
5-3. HeNe（R）(633nm） OFF

6. Turn mercury lamp power to OFF.

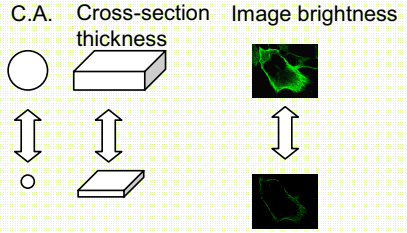
Annex 1

■ ■ Relationship between confocal principle and tuning mechanism ■ ■

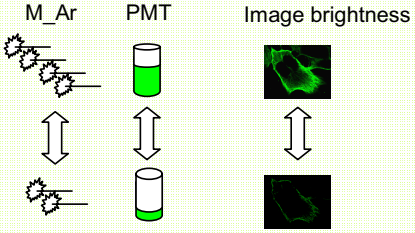


■ Memo ■

Relationship between confocal aperture and optical cross-section, image brightness

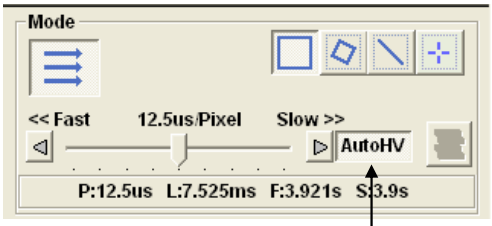


Relationship between laser output/ detector sensitivity and image brightness



Annex 2

■ ■ Image acquisition of less noises ■ ■



AutoON: The slower it is set, the more noise can be removed by keeping current image brightness.
AutoOFF: The slower it is set, the more it is getting brighter and noise is also removed.

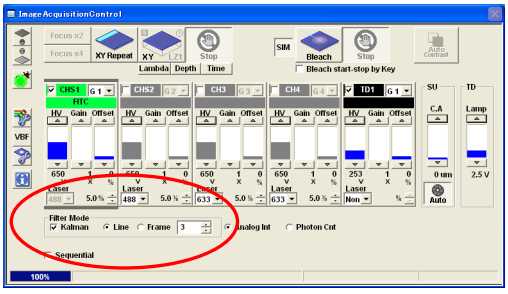
Method to make scanning speed slower

Set scanning speed slower so that an image can be acquired without detecting noises from the beginning.

Merit:
● With Kalman integration, comparatively sharp image can be acquired.
Demerit:
● Speed of scanning at one time is slow.

1. Select ScanSpeed.

****



Method to use Kalman Integration

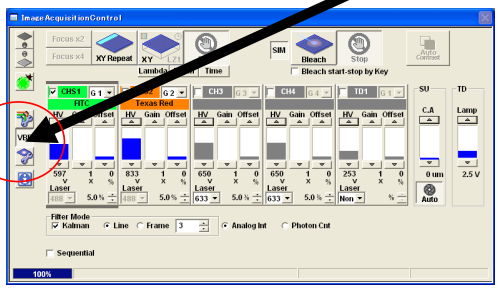
Images are averaged while image is being acquired for number of times specified. As the results, noises are also averaged so that roughness of whole image can be suppressed.
Merit:
● Speed of scanning at one time is fast.
Demerit:
● Images are averaged so that an image may get dim, more or less.

1. Click Kalman and select Line or Frame.
2. Enter number of integration times for the image (number of scanning cycles).

Annex 3

■ ■ Method to change width or position of slit in manual mode ■ ■

1

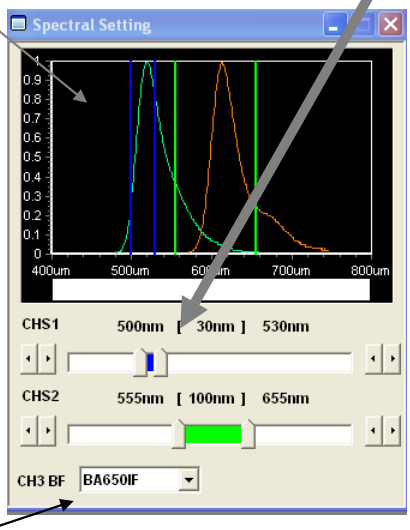


1. Click VBF button and display SpectralSetting window.

2. Using or , move left or right.

2

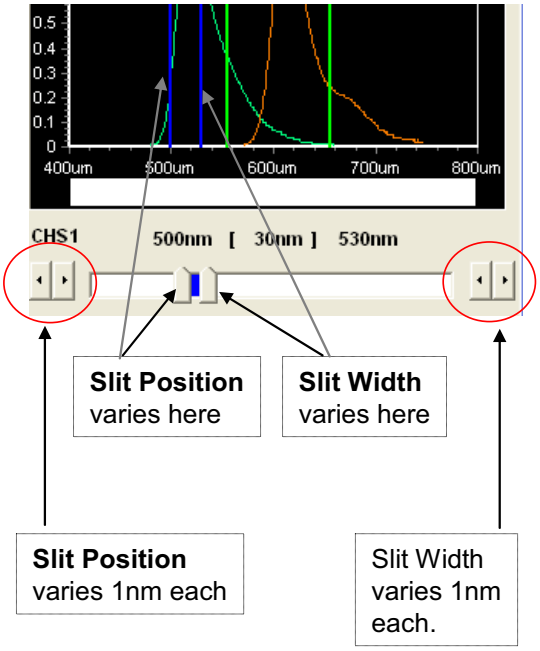
Spectral of fluorescent dye selected and Slit width and position are displayed.



CH1 Slit Adj

Ch2 Slit Adj

Ch3 Fluorescent filter selection


CHS1 500nm [30nm] 530nm
Slit Position varies here
Slit Width varies here
Slit Position varies 1nm each
Slit Width varies 1nm each.

∗ Numeric can be entered.

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